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(54) Title: PHARMACEUTICAL COMPOSITION CONTAINING HISTONE DEACETYLASE INHIBITOR

(57) Abstract: An anticancer drug having a synergistic effect by combined use of a histone acetylase derivative such as N-(2-aminophenyl)-4-[N-(pyridin-3-ylmethoxycarbonyl) aminomethyl]benzamide (MS-275) and another anticancer active substance.

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DESCRIPTION

PHARMACEUTICAL COMPOSITION CONTAINING HISTONE DEACETYLASE INHIBITOR

TECHNICAL FIELD

The present invention relates to a pharmaceutical composition or drug combination for treatment of cancer comprising a histone deacetylase inhibitor and another anticancer active substance.

BACKGROUND ART

At the present time, cancer is the first leading cause of death. Up until now, many researchs on cancer have been conducted and tremendous money and time have been spended on these researchs. However, despite research in methods of treatment spanning diverse fields such as surgery, radiotherapy, and thermotherapy, cancer has not been overcome. Among these, chemotherapy is a major sector and many anticancer drugs have been researched. For example, as chemotherapy drugs for cancer, cisplatin, etoposide, 5-fluorouracil, gemcitabine, paclitaxel, docetaxel, carboplatin, oxaliplatin, doxorubicin, vinblastin, etc. have been used.

Japanese Unexamined Patent Publication (Kokai) No. 10-152462 discloses a benzamide derivative. The following fact is disclosed; said benzamide derivative has a differentiation inducing action, is useful as a pharmaceutical for the treatment or alleviation of malignant tumors, autoimmune diseases, skin diseases, and parasitic infection, is particularly effective as an anticancer drug, and is effective against hematopoietic cancers and solid cancers.

Patent Document 1

Japanese Unexamined Patent Publication (Kokai) No. 10-152462

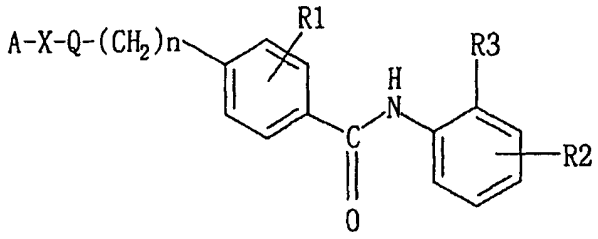
DISCLOSURE OF THE INVENTION

However, anticancer drugs have limitation at a dosage of a single drug due to their strong toxicity to normal cells. Except for some cancers, treatment by administration of a single drug is not enough to achieve a sufficient efficacy.

The present invention was made to reduce the toxicity posing a problem in current chemotherapy and achieve a high treatment effect.

Accordingly, the present invention provides a pharmaceutical composition or combination as active ingredients comprising:

(a) at least one of the benzamide derivatives represented by formula (1):



(1)

wherein A is an optionally substituted phenyl group or an optionally substituted heterocyclic group wherein the substituent(s) for the phenyl group or the heterocyclic group is (are) 1 to 4 substituents selected from the group consisting of a halogen atom, a hydroxyl group, an amino group, a nitro group, a cyano group, an alkyl group having 1 to 4 carbons, an alkoxy group having 1 to 4 carbons, an aminoalkyl group having 1 to 4 carbons, an alkylamino group having 1 to 4 carbons, an acyl group having 1 to 4 carbons, an acylamino group having 1 to 4 carbons, an alkylthio group having 1 to 4 carbons, a perfluoroalkyl group having 1 to 4 carbons, a perfluoroalkyloxy group having 1 to 4 carbons, a carboxyl group, an alkoxycarbonyl group having 1 to 4 carbons, a phenyl group and a heterocyclic group;

X is a bond or a moiety having a structure selected from those illustrated in formula (2):

$-(CH_2)e-$, $-(CH_2)g-O-(CH_2)e-$,

$-(CH_2)g-N(R4)-(CH_2)e-$, $-(CH_2)g-S-(CH_2)e-$,

(2)

$-(CH_2)g-C(=O)-(CH_2)m-$, $-(CH_2)g-N(R5)-C(=O)-(CH_2)m-$,

$-(CH_2)g-C(=O)-N(R5)-(CH_2)m-$

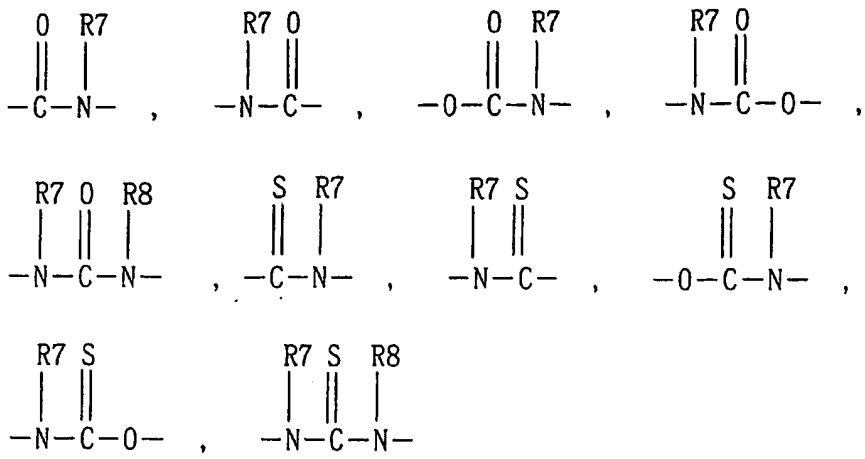
wherein e is an integer of 1 to 4; g and m are independently an integer of 0 to 4; R4 is a hydrogen atom or an optionally substituted alkyl group having 1 to 4 carbons, or the acyl group represented by formula (3)

$-C(=O)-R6$ (3)

wherein R6 is an optionally substituted alkyl group having 1 to 4 carbons, a perfluoroalkyl group having 1 to 4 carbons, a phenyl group or a heterocyclic group; R5 is a hydrogen atom or an optionally substituted alkyl group having 1 to 4 carbons;

n is an integer of 0 to 4, provided that when X is a bond, n is not zero;

Q is a moiety having a structure selected from those illustrated in formula (4)



(4)

wherein R7 and R8 are independently a hydrogen atom or an optionally substituted alkyl group having 1 to 4 carbons;

R1 and R2 are independently a hydrogen atom, a halogen atom, a hydroxyl group, an amino group, an alkyl group having 1 to 4 carbons, an alkoxy group having 1 to 4 carbons, an aminoalkyl group having 1 to 4 carbons, an alkylamino group having 1 to 4 carbons, an acyl group having 1 to 4 carbons, an acylamino group having 1 to 4 carbons, an alkylthio group having 1 to 4 carbons, a perfluoroalkyl group having 1 to 4 carbons, a perfluoroalkyloxy group having 1 to 4 carbons, a carboxyl group or an alkoxycarbonyl group having 1 to 4 carbons;

R3 is a hydroxyl group or amino group or a pharmaceutically acceptable salt thereof as HDAC inhibiting substance, and

(b) at least one substance as another anti-cancer active substance selected from a group consisting of cisplatin, etoposide, camptothecin, 5-fluorouracil, gemcitabine, paclitaxel, docetaxel, carboplatin, oxaliplatin, doxorubicin and vinblastin.

The present invention further provides a cancer treatment kit comprising a pharmaceutical combination, which comprises:

(i) at least one of said ingredients (a) which is a

histone deacetylase inhibiting substance,

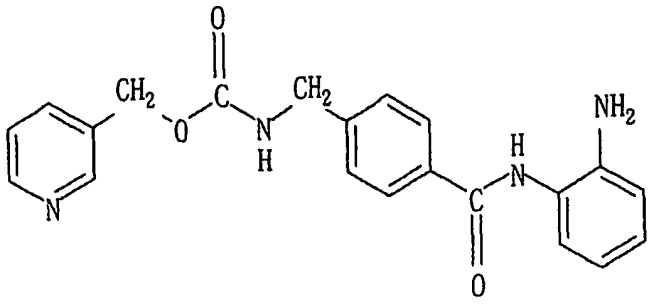
(ii) at least one of said ingredients (b) which is another anti-cancer active substance, and

(iii) an instruction for administration schedule for simultaneous or sequential administration according to a kind of cancer (for sequential administration to a patient at periodic intervals).

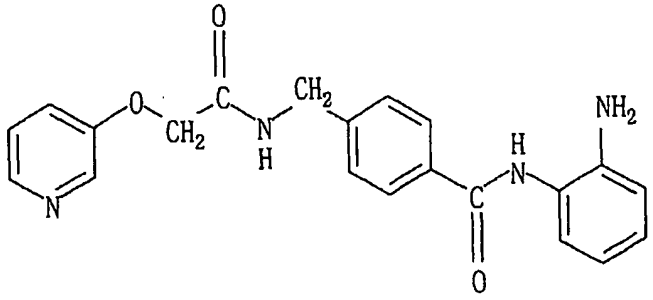
The "pharmaceutical combination" in the present invention means a combination of an ingredient (a) which is a histone deacetylase inhibiting substance and an ingredient (b) which is another anti-cancer active substance, wherein the ingredient (a) and the ingredient (b) are administered simultaneously or at different times (or sequentially).

The present invention includes a method of treatment of cancer comprising administering said ingredient (a) and said ingredient (b) to patients simultaneously or at different times (or sequentially). In this situation, an administration sequence of said ingredient (a) and said ingredient (b) is appropriately selected according to a kind of cancer and kinds of said ingredient (a) and said ingredient (b). Further, the present invention also includes use of said ingredient (a) and said ingredient (b) for producing a pharmaceutical composition or drug combination of the present invention for treating cancer and use of said ingredient (a) and said ingredient (b) for producing the kit of the present invention.

The benzamide derivative which is a histone deacetylase inhibiting substance or pharmaceutically acceptable salts thereof is preferably selected from represented by the following formulas (5) to (8):



(5)



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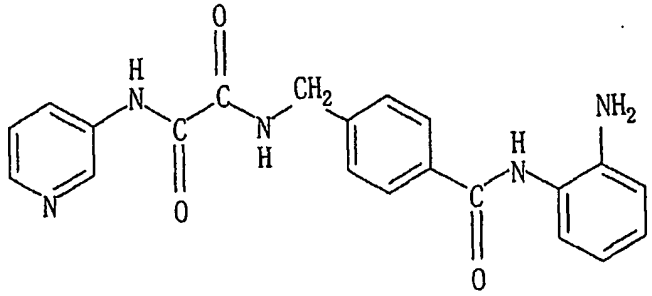

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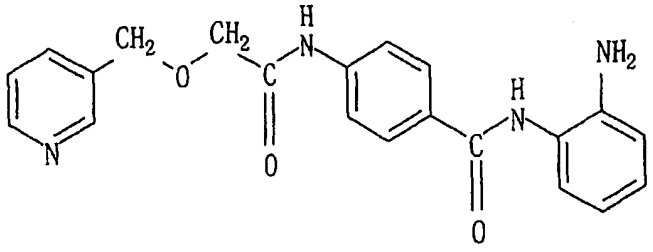
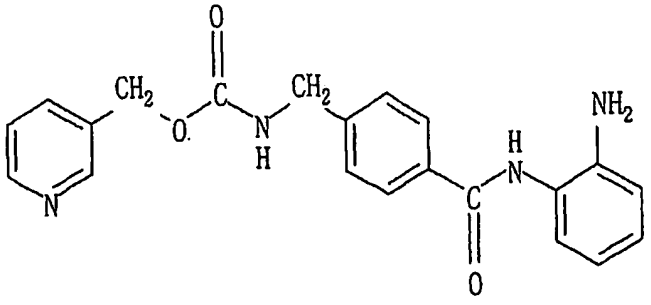


(8)

More preferably, the benzamide derivative is represented by the following formula (5) or pharmaceutically acceptable salt thereof:



(5)

In the pharmaceutical combination or composition in the present invention, said ingredient (b) which is another anti-cancer active substance is preferably cisplatin, more preferably the combination or composition which is for treatment of colon cancer, non-small cell lung cancer, ovarian cancer or pancreatic cancer.

Further, in the pharmaceutical combination or composition in the present invention, said ingredient (b) which is another anti-cancer active substance is preferably etoposide, more preferably the combination or composition which is for treatment of ovarian cancer.

Further, in the pharmaceutical combination or composition in the present invention, said ingredient (b) which is another anti-cancer active substance is preferably camptothecin, more preferably the combination or composition which is for treatment of colon cancer, non-small cell lung cancer, ovarian cancer or pancreatic cancer.

Further, in the pharmaceutical combination or composition in the present invention, said ingredient (b) which is another anti-cancer active substance is preferably 5-fluorouracil, more preferably the combination or composition which is for treatment of breast cancer or colon cancer.

Further, in the pharmaceutical combination or composition in the present invention, said ingredient (b) which is another anti-cancer active substance is preferably gemcitabine, more preferably the combination or composition which is for treatment of non-small cell lung cancer, colon cancer or ovarian cancer.

Further, in the pharmaceutical combination or composition in the present invention, said ingredient (b) which is another anti-cancer active substance is preferably paclitaxel, more preferably the combination or composition which is for treatment of breast cancer, prostate cancer or ovarian cancer.

Further, in the pharmaceutical combination or

composition in the present invention, said ingredient (b) which is another anti-cancer active substance is preferably docetaxel, more preferably the combination or composition which is for treatment of non-small cell lung cancer, ovarian cancer, pancreatic cancer or prostate cancer.

Further, in the pharmaceutical combination or composition in the present invention, said ingredient (b) which is another anti-cancer active substance is preferably carboplatin, more preferably the combination or composition which is for treatment of non-small cell lung cancer, ovarian cancer or pancreatic cancer.

Further, in the pharmaceutical combination or composition in the present invention, said ingredient (b) which is another anti-cancer active substance is preferably oxaliplatin, more preferably the combination or composition which is for treatment of colon cancer or ovarian cancer.

Further, in the pharmaceutical combination or composition in the present invention, said ingredient (b) which is another anti-cancer active substance is preferably doxorubicin, more preferably the combination or composition which is for treatment of ovarian cancer.

Further, in the pharmaceutical combination or composition in the present invention, said ingredient (b) which is another anti-cancer active substance is preferably vinblastin, more preferably the combination or composition which is for treatment of non-small cell lung cancer.

Further, the pharmaceutical combination in the present invention is preferable, of which said ingredient (a) which is histone deacetylase inhibiting substance and said ingredient (b) which is another anti-cancer active substance are sequentially administered to patients.

Of the pharmaceutical combination, said ingredient (b) which is another anti-cancer active substance is preferably paclitaxel. As the administration sequence

thereof, it is preferable to administer paclitaxel and then said ingredient (a) which is a histone deacetylase inhibiting substance. The pharmaceutical combination for treatment of breast cancer or ovarian cancer is more preferable.

Further, of the pharmaceutical combination, said ingredient (b) which is another anti-cancer active substance is preferably cisplatin. As the administration sequence thereof, it is preferable to administer said ingredient (a) which is a histone deacetylase inhibiting substance, and then cisplatin. The pharmaceutical combination for treatment of non-small cell lung cancer is more preferable. Or, the administration sequence thereof is preferably cisplatin, and then said ingredient (a) which is a histone deacetylase inhibiting substance. The pharmaceutical combination for treatment of colon cancer, non-small cell lung cancer, ovarian cancer or pancreatic cancer is more preferable.

Further, of the pharmaceutical combination, said ingredient (b) which is another anti-cancer active substance is preferably gemcitabine. As the administration sequence thereof, it is preferable to administer said ingredient (a) which is a histone deacetylase inhibiting substance, and then gemcitabine. The pharmaceutical combination for treatment of non-small cell lung cancer is more preferable. Or, the administration sequence thereof is preferably gemcitabine, and then said ingredient (a) which is a histone deacetylase inhibiting substance. The pharmaceutical combination for treatment of colon cancer, non-small cell lung cancer, ovarian cancer or pancreatic cancer is more preferable.

Further, of the pharmaceutical combination, said ingredient (b) which is another anti-cancer active substance is preferably docetaxel. As the administration sequence thereof, it is preferable to administer docetaxel, and then said ingredient (a) which is a

histone deacetylase inhibiting substance. The pharmaceutical combination for treatment of non-small cell lung cancer, ovarian cancer, pancreatic cancer or prostate cancer is more preferable.

Further, of the pharmaceutical combination, said ingredient (b) which is another anti-cancer active substance is preferably carboplatin. As the administration sequence thereof, it is preferable to administer carboplatin, and then said ingredient (a) which is a histone deacetylase inhibiting substance. The pharmaceutical combination for treatment of non-small cell lung cancer, ovarian cancer, pancreatic cancer or prostate cancer is more preferable.

Further, of the pharmaceutical combination, said ingredient (b) which is another anti-cancer active substance is preferably oxaliplatin. As the administration sequence thereof, it is preferable to administer oxaliplatin, and then said ingredient (a) which is a histone deacetylase inhibiting substance. The pharmaceutical combination for treatment of colon cancer or ovarian cancer is more preferable.

Further, of the pharmaceutical combination, said ingredient (b) which is another anti-cancer active substance is preferably doxorubicin. As the administration sequence thereof, it is preferable to administer doxorubicin, and then said ingredient (a) which is a histone deacetylase inhibiting substance. The pharmaceutical combination for treatment of ovarian cancer is more preferable.

Further, of the pharmaceutical combination, said ingredient (b) which is another anti-cancer active substance is preferably vinblastin. As the administration sequence thereof, it is preferable to administer vinblastin, and then said ingredient (a) which is a histone deacetylase inhibiting substance. The pharmaceutical combination for treatment of non-small cell lung cancer is more preferable.

Further, of the pharmaceutical combination, said ingredient (b) which is another anti-cancer active substance is preferably 5-fluorouracil. As the administration sequence thereof, it is preferable to administer 5-fluorouracil, and then said ingredient (a) which is a histone deacetylase inhibiting substance. The pharmaceutical combination for treatment of colon cancer is more preferable.

In the pharmaceutical composition of the present invention, said ingredient (a) and said ingredient (b) may be made into the pharmaceutical composition using compound per se which are these active ingredients, may be made into the pharmaceutical composition using a preparation containing said ingredient (a) as an active ingredient and a preparation containing said ingredient (b) as an active ingredient, or may be made into the pharmaceutical composition using the compound per se which is either of said ingredient (a) or said ingredient (b) and a preparation of the other prepared in advance. And, in the pharmaceutical combination of the present invention, usually separately prepared preparations, that is, a preparation containing said ingredient (a) as an active ingredient and a preparation containing said ingredient (b) as an active ingredient, are administered simultaneously or at a different time (or consecutively).

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is a graph showing the principle of judgment of the existence of a synergistic action.

BEST MODE FOR CARRYING OUT THE INVENTION

The present invention relates to a pharmaceutical composition or combination comprising a benzamide derivative represented by formula (1) which is a histone deacetylase inhibiting substance and another anticancer active substance.

As used herein, "1 to 4 carbons" means a carbon number per a single substituent; for example, for dialkyl substitution it means 2 to 8 carbons.

A heterocycle in the compound represented by formula (1) is a monocyclic heterocycle having 5 or 6 members containing 1 to 4 nitrogen, oxygen or sulfur atoms or a bicyclic-fused heterocycle. The monocyclic heterocycle includes pyridine, pyrazine, pyrimidine, pyridazine, thiophene, furan, pyrrole, pyrazole, isoxazole, isothiazole, imidazole, oxazole, thiazole, piperidine, piperazine, pyrrolidine, quinuclidine, tetrahydrofuran, morpholine, thiomorpholine and the like. The bicyclic fused heterocycle includes quinoline; isoquinoline; naphthyridine; fused pyridines such as furopyridine, thienopyridine, pyrrolopyridine, oxazolopyridine, imidazolopyridine and thiazolopyridine; benzofuran; benzothiophene; benzimidazole and the like. A halogen may be fluorine, chlorine, bromine or iodine. An alkyl having 1 to 4 carbons includes methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl and tert-butyl.

An alkoxy having 1 to 4 carbons includes methoxy, ethoxy, n-propoxy, isopropoxy, allyloxy, n-butoxy, isobutoxy, sec-butoxy, tert-butoxy and the like.

An aminoalkyl having 1 to 4 carbons includes aminomethyl, 1-aminoethyl, 2-aminopropyl and the like. An alkylamino having 1 to 4 carbons includes N-methylamino, N,N-dimethylamino, N,N-diethylamino, N-methyl-N-ethylamino, N,N-diisopropylamino and the like. An acyl having 1 to 4 carbons includes acetyl, propanoyl, butanoyl and like. An acylamino having 1 to 4 carbons includes acetylamino, propanoylamino, butanoylamino and the like. An alkylthio having 1 to 4 carbons includes methylthio, ethylthio, propylthio and the like. A perfluoroalkyl having 1 to 4 carbons includes trifluoromethyl, pentafluoroethyl and the like. A perfluoroalkyloxy having 1 to 4 carbons includes trifluoromethoxy, pentafluoroethoxy and the like. An alkoxycarbonyl having 1 to 4 carbons includes methoxycarbonyl and ethoxycarbonyl. An optionally substituted alkyl having 1 to 4 carbons includes methyl,

ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl and tert-butyl and these having 1 to 4 substituents selected from the group consisting of a halogen, hydroxyl, amino, nitro, cyano, phenyl and a heterocycle.

A pharmaceutically acceptable salt of ingredient (a) as histone deacetylase inhibiting substance of this invention includes salts with an inorganic acid such as hydrochloric acid, hydrobromic acid, sulfuric acid and phosphoric acid; and with an organic acid such as acetic acid, lactic acid, tartaric acid, malic acid, succinic acid, fumaric acid, maleic acid, citric acid, benzoic acid, trifluoroacetic acid, p-toluenesulfonic acid and methanesulfonic acid.

The ingredient (a) which is a histone deacetylase inhibiting substance of this invention may be produced in accordance with the process of Japanese unexamined patent publication (Kokai) No. 10-152462. And, the ingredient (b) which is another anti-cancer active substance is commercially available or can be produced by known methods.

The pharmaceutical composition or combination of this invention is useful for cancer treatment. The composition itself may be used in the form of a general pharmaceutical formulation. And of the combination the ingredients (a) and (b) may be used in the form of a general pharmaceutical formulation.

The pharmaceutical composition comprising the active ingredient (a) and (b) is prepared with a generally used diluent or excipient such as filler, extender, binder, moisturizing agent, disintegrator, surfactant and lubricant. And the pharmaceutical combination is prepared by independent active ingredients, with a generally used diluent or excipient such as filler, extender, binder, moisturizing agent, disintegrator, surfactant and lubricant. The pharmaceutical formulation may have a variety of dosage forms such as tablet, pill, powder, solution, suspension, emulsion, granule, capsule,

injection (e.g., solution, suspension) and suppository.

For preparing tablets, a variety of carriers well-known in the art may be used. Such a carrier includes excipients such as lactose, glucose, starch, calcium carbonate, kaoline, crystalline cellulose and silicic acid; binders such as water, ethanol, propanol, simple syrup, glucose solution, starch solution, gelatin solution, carboxymethyl cellulose, shellac, methyl cellulose and polyvinyl pyrrolidone; disintegrators such as dried starch, sodium alginate, powdered agar, calcium carmelose, starch and lactose; disintegration retarders such as sucrose, cocoa butter and hydrogenated oil; absorption promoters such as quaternary ammonium base and sodium lauryl sulfate; moisturizing agents such as glycerin and starch; adsorbents such as starch, lactose, kaoline, bentonite, colloidal silicic acid; and glidants such as talc, stearates and polyethylene glycol. The tablet may be, if necessary, one coated with a common coating; for example, sugar-coated tablet, gelatin-coated tablet, enteric coated tablet, film-coated tablet, double-layer tablet and multilayer tablet.

In forming pills, a variety of carriers well-known in the art may be used. Such a carrier includes excipients such as crystalline cellulose, lactose, starch, hydrogenated vegetable oil, kaoline and talc; binders such as powdered acacia, powdered tragacanth gum and gelatin; disintegrators such as calcium carmelose and agar.

Capsule may be prepared by blending an active ingredient with a variety of the above carriers as usual and filling the resulting blend into, for example, a hard or soft gelatin capsule or the like.

For preparing injection, solution, emulsion and suspension are sterilized and preferably isotonic with blood. It may be prepared using diluents commonly used in the art; for example, water, ethanol, macrogol, propylene glycol, ethoxylated isostearyl alcohol, polyoxyisostearyl

alcohol and polyoxyethylene sorbitan fatty acid esters. The pharmaceutical preparation may contain sodium chloride necessary to prepare an isotonic solution, glucose or glycerin, as well as usual solubilizers, buffers and soothing agents.

Suppository may be formed using a variety of well-known carriers; for example, semi-synthetic glyceride, cocoa butter, higher alcohols, higher alcohol esters and polyethylene glycol.

Furthermore, the pharmaceutical formulation may contain coloring agents, preservatives, perfumes, flavors, sweeteners and/or other drugs.

The volume ratio of the active ingredients (b) to (a) to be included in the pharmaceutical composition of the present invention is not limited and is appropriately selected from a broad range of the volume ratios. In the case of cisplatin, the molar ratio is 0.001 to 10000, preferably 0.01 to 1000, to 1 of the benzamide derivative (said ingredient (a)). In the case of etoposide, the molar ratio is 0.001 to 10000, preferably 0.01 to 1000, to 1 of the benzamide derivative.

In the case of camptothecin, the molar ratio is 0.00001 to 10, preferably 0.0001 to 1, to 1 of the benzamide derivative (said ingredient (a)). In the case of 5-fluorouracil, the molar ratio is 0.01 to 100000, preferably 0.1 to 10000, to 1 of the benzamide derivative. In the case of gemcitabine, the molar ratio is 0.00001 to 100, preferably 0.0001 to 10, to 1 of the benzamide derivative (said ingredient (a)). In the case of paclitaxel, the molar ratio is 0.000001 to 0.01, preferably 0.00001 to 0.001, to 1 of the benzamide derivative (said ingredient (a)).

In the case of docetaxel, the molar ratio is 0.0000001 to 1, preferably 0.000001 to 0.1, to 1 of the benzamide derivative (said ingredient (a)).

In the case of carboplatin, the molar ratio is 0.001 to 10000, preferably 0.01 to 1000, to 1 of the benzamide

derivative (said ingredient (a)).

In the case of oxaliplatin, the molar ratio is 0.001 to 10000, preferably 0.01 to 1000, to 1 of the benzamide derivative (said ingredient (a)).

In the case of doxorubicin, the molar ratio is 0.000001 to 1, preferably 0.00001 to 0.1, to 1 of the benzamide derivative (said ingredient (a)).

In the case of vinblastin, the molar ratio is 0.000001 to 1, preferably 0.00001 to 0.1, to 1 of the benzamide derivative (said ingredient (a)).

An administration route of the pharmaceutical composition or combination is not limited, and selected depending on their dosage form, patient's age, sex, severity of disease and other conditions. For example, tablet, pill, solution, suspension, emulsion, granule and capsule may be orally administered; injection may be intravenously administered solely or in combination with a common infusion fluid such as glucose, amino acids and the like, or if necessary, intramuscularly, subcutaneously or intraperitoneally as a sole preparation. Suppository may be intrarectally administered.

Dose of the pharmaceutical composition or combination of this invention may be selected, depending on their dosage form, patient's age, sex and severity of disease, and other conditions, as appropriate, and the amount of the active ingredients in the composition may be generally about 0.0001 to 1000 mg/kg a day. It is preferable that a unit dosage form may contain about 0.001 to 1000 mg of the active ingredient(s).

Further, in the case of pharmaceutical combinations, the amount of the active ingredient of the benzamide derivative (said ingredient (a)) may be about 0.0001 to 1000 mg per kg body weight. In the case of cisplatin, the amount may be about 0.01 to 50 mg per kg body weight. In the case of etoposide, the amount may be about 0.1 to 10 mg per kg body weight. In the case of camptothecin, the

amount may be about 0.1 to 10 mg per kg body weight.

In the case of 5-fluorouracil, the amount may be about 0.1 to 200 mg per kg body weight.

In the case of gemcitabine, the amount may be about 1 to 300 mg per kg body weight. In the case of paclitaxel, the amount may be about 0.1 to 100 mg per kg body weight.

In the case of docetaxel, the amount may be about 0.1 to 50 mg per kg body weight.

In the case of carboplatin, the amount may be about 0.2 to 100 mg per kg body weight.

In the case of oxaliplatin, the amount may be about 0.1 to 50 mg per kg body weight.

In the case of doxorubicin, the amount may be about 0.1 to 50 mg per kg body weight.

In the case of vinblastin, the amount may be about 0.01 to 5 mg per kg body weight.

For administration of pharmaceutical combinations, in the case of simultaneous administration, the first active ingredient and the second active ingredient are administered without any time interval. In the case of administration at different times (consecutively), it is preferable to administer the first active ingredient and then administer the second active ingredient half a day to 60 days later.

EXAMPLES

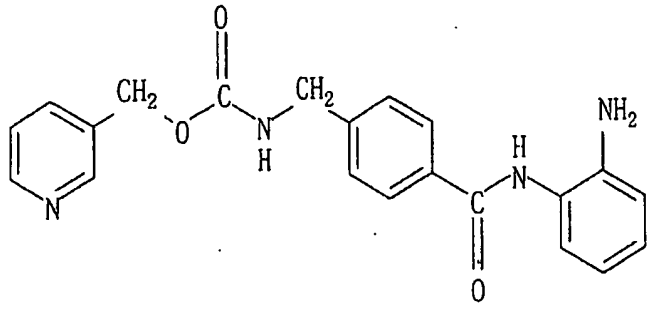
Next, the present invention will be explained with examples more specifically.

Examples. Confirmation of Synergistic Effect Between Histone Deacetylase Inhibitor and Known Anticancer Active Substances on Cancer Cell Proliferation

The synergistic effects in combined use of the histone deacetylase inhibitor of the present invention and various types of known anticancer active substances on various types of cancer cell lines were confirmed by the examples.

Test Substances

As the histone deacetylase inhibitor of the present invention, N-(2-aminophenyl)4-[N-(pyridin-3-ylmethoxycarbonyl)aminomethyl]benzamide (MS-275) represented by the following formula (5) was used.



(5)

And, as known anticancer activity substances used in conjunction with the above MS-275 compound, paclitaxel (PTX), camptothecin (CPT), etoposide (VP-16), cisplatin (CDDP), gemcitabine (GEM), 5-fluorouracil (5-FU), docetaxel (DTX), carboplatin (CBDCA), oxaliplatin (OXP), doxorubicin (DOX), or vinblastin (VBL) was used.

Tested Cancer Cells

As the tested cancer cells, the following cell lines were used:

Colon cancer cell line: HT-29 and/or HCT116;

Non-small cell lung cancer cell line: NCI-H522, A549, Calu-1, Calu-3, NCI-H23, and/or NCI-H460;

Ovarian cancer cell line: SK-OV-3 and/or OVCAR-3;

Pancreatic cancer cell line: PANC-1 and/or Capan-1;

Breast cancer cell line: PC-3 and/or LNcaP.

Methods of Combined Use

In experiments, to evaluate the combined effect of the MS-275 which is a histone deacetylase inhibitor and another known anticancer active substance, (i) effects of the MS-275 alone, (ii) effects of another known anticancer active substance, and (iii) effects from combined use of the MS-275 and another anticancer active substance were measured. For the measurement of the effects of (iii), the following two types of methods were used.

Simultaneously Combined Use:

In this method, the test cancer cells were incubated for 72 to 120 hours in a medium containing a mixture of MS-275 and another known anticancer active substance, and then the surviving cancer cells were measured.

Consecutively Combined Use:

In this method, the test cancer cells were incubated for 24 hours in a medium containing one of the test substances, and the medium containing said test substance was aspirated at this point of time. Then the cells were incubated for 24 hours in a medium containing the other of the test substances, the medium containing said test substance was aspirated at this point of time, then the cells were incubated for another 72 hours in a medium not containing the test substances, and then the surviving cancer cells were measured. In the consecutively combined use, the MS-275 was made to act in the first 24 hours and the other known anticancer active substance was made to act in the succeeding 24 hours. And in the reversed order of what was made to act this experiment was performed. Further, in the single administration control for the combined use, the test substance was made to act in only the initial 24 hours or the succeeding 24 hours. In another 24 hour period and the final 72 hours, the cells were incubated in the absence of the test substance, and then the surviving cancer cells were measured.

Method of Measurement of Surviving Cancer Cells

After the above treatment (incubation) of the cancer cells by the test substances was ended, the surviving cells were measured by one of the following two methods.

Neutral Red Assay:

In this measurement method the following property is utilized; only surviving cells can take a water soluble dye, Neutral Red, into the cells. The above treatment of cancer cells by the test substance was performed in wells. A Neutral Red solution (1 mg/ml in PBS) was added into the wells after the end of the treatment (incubation). The incubation at 37°C for one hour allowed

the Neutral Red to be taken into the cells. The solution was aspirated and 100% ethanol and 0.1M NaH_2PO_4 were added to the wells. The Neutral Red taken into the cells was extracted from the cells and then the extracted Neutral Red was measured by a microplate reader at 540 nm.

MTS Assay:

This method is to investigate cell survivability by utilizing the fact that MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenol)-2-(4-sulfonyl)-2H-tetrazoliumm) is metabolized to formazan by mitochondria dehydrogenase existing in surviving cells. In this method the experiment was performed using a Cell Titer 96 (trademark) aqueous one solution cell proliferation assay of Promega in accordance with the instructions attached to the reagents.

Combined Ratio of Test Substances and Judgment of Synergism

The combined ratio of the test substances was determined as follows: In the graph of FIG. 1, the abscissa shows the log (Log M) of the concentrations of the test substances, and the ordinate shows the relative survival rate in the case indexed to the surviving tested cancer cells in the case of zero concentration of test substances. Graphs of the concentration of the test substances and the relative survival rate of the tested cancer cells in the case of the test substances alone were made. The concentrations of the test substances in the case of relative survival rates of 50%, IC_{50}, were calculated.

Regarding the IC_{50}'s of the test substances A and B for which the existence of a synergistic effect was desired to be learned, in the case that the IC_{50} of the test substance A was 1 μM and 0.01 μM as the IC_{50} of the test substance B was 0.01 μM, since the anticancer effect of the test substance B was 100 times that of the test

substance A, the combined ratio of the test substance A and test substance B was made 100:1. This ratio was kept constant across the various total concentrations of the test substances. However, the IC_{50} of a test substance differed according to the tested cancer cells, so the combined ratio needed to be determined for each test substance and for each type of tested cancer cells.

In FIG. 1, the "concentration-survival rate curve" of the test substance A was shown in a solid line, and the "concentration-survival rate curve" of the test substance B was shown in a dotted line. Further, given that the test substance A and test substance B were used in a constant ratio (for example, 100:1) and at various total concentrations and that the combined effect of the test substances was "additive", a "concentration-survival rate curve" could be drawn for the case of combined use by calculation. For example, in FIG. 1, this could be shown in a series of black dots.

On the other hand, an actual "concentration-survival rate curve" could be drawn by calculating from the actually measured values in the case of use of the test substance A and test substance B at a constant ratio (for example, 100:1) but at various total concentrations. When the curve is present at the left side from the "concentration-survival rate curve" drawn by calculation under the assumption of "additive" as shown for example by a series of black squares in FIG. 1, the combined effects of the test substance A and the test substance B were judged to be "synergistic". Meanwhile, when the actual "concentration-survival rate curve" was drawn at the right side from the "concentration-survival rate curve" drawn by calculation under the assumption of "additive" as shown for example by a series of black triangles in FIG. 1, the combined effects of the test substance A and the test substance B were judged to be "antagonistic".

In actuality, the combination index (CI) was

calculated from the measurement results by the method described in Chou TC et al., *Adv. Enzyme Regul. 22*: 27-55 (1984) (Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors). In this case, when the combined effects of the test substance A and test substance B were additive, CI=1. When CI was less than 1, the effects were synergistic. When CI was more than one, the effects were antagonistic. Further, the following were judged; the smaller a value less than 1 was the higher the "synergism" was. And the greater a value more than 1 was, the higher the "antagonism" was.

Further, the relationship between the range of the CI value and the degree of synergism and antagonism is expressed as follows:

Table 1

Range of CI value	Symbol	Description
<0.1	+++++	Very strongly synergistic
0.1 to 0.3	++++	Strongly synergistic
0.3 to 0.7	+++	Synergistic
0.7 to 0.85	++	Moderately synergistic
0.85 to 0.9	+	Slightly synergistic
0.9 to 1.1	±	Additive
1.1<	-	Antagonistic

RESULTS

The ratios between MS-275 and other anticancer active substances with respect to each tested cancer cell line in the case of simultaneous combined use are as follows:

Table 2

Ratio of MS-275 and Other Anticancer Active Substances (X) in Simultaneous Combined Use

Cancer cell line		Time (hr)	Ratio (MS-275:X)					
			PTX	CPT	VP-16	CDDP	GEM	5-FU
Colon cancer	HT-29	72		30:1		1:5	5:1	1:10
	HCT116	72		50:1	1:1	1:10	100:1	1:10
Non-small cell lung cancer	NCI-H522	72 120	200:1 400:1				500:1 2000:1	
	A549	72	100:1			1:10	40:1	
Ovarian cancer	SK-OV-3	72 120	1000:1 1000:1	100:1 100:1	1:1 1:1	1:2 1:2		
	OVCAR-3	120	1000:1	100:1	4:1	1:1	200:1	
Pan-creatic cancer	PANC-1	72 120	2000:1 2000:1	200:1 400:1		1:1 1:1	200:1 200:1	1:1 1:1
Breast cancer	MCF-7	72 120	400:1 400:1					1:10 1:10
Prostate cancer	PC-3	72 120	100:1 10:1		1:40 1:50			

The results in the case of simultaneous combined use are as follows:

Table 3

Synergistic Effect in Combined Use of MS-275 and Other Anticancer Active Substances in Simultaneous Combined Use

Cancer cell line		Time (hr)	Other anticancer active substance					
			PTX	CPT	VP-16	CDDP	GEM	5-FU
Colon cancer	HT-29	72		-		-	-	-
		72						+++
	HCT116	72		-	-	-	-	-
Non-small cell lung cancer	NCI-H522	72	-				±	
		120	-				-	
		72				±		
	A549	72	-			-	-	
		72				+++		
	Calu-1	72				+++		
	Calu-3	72				+++		
	A-427	72				-		
	NCI-H23	72				+++		
	NCI-H358	72				±		
	NCI-H460	72				+++		
Ovarian cancer	SK-OV-3	72	-	-	+++	++		
		120	-	-	±	-		
	OVCAR-3	120	-	-	-	-	-	
Pan-creatic cancer	PANC-1	72	-	+++		++	+++	-
		120	-	-		++	-	-
Breast cancer	MCF-7	72						+++
		120	-					++
Pro-state cancer	PC-3	72	-		-			
		120	++		-			

As explained above, the combined effects of MS-275 and another known anticancer drug PTX, CPT, VP-16, GEM, or 5-FU were detected in specific cancer cells. Further, the combined effects of MS-275 and CDDP were detected in a broad range of cancer cells.

Further, the results in the case of consecutive combined use are shown in Table 4 (combined use of MS-275 and PTX), Table 5 (combined use of MS-275 and GEM), Table 6 (combined use of MS-275 and CDDP), Table 7 (combined use of MS-275 and CPT), Table 8 (combined use of MS-275 and DTX), Table 9 (combined use of MS-275 and CBDCA), Table 10 (combined use of MS-275 and OXP), Table 11 (combined use of MS-275 and DOX), Table 12 (combined use of MS-275 and VBL), and Table 13 (combined use of MS-275

and 5-FU). Note that in these tables, "Ratio 275:XS" means the ratio of MS-275 and another anticancer active substance (X), while "275->X->f" indicates treatment by MS-275 in the initial treatment period of 24 hours, treatment by another anticancer active substance in the following treatment period of 24 hours, then incubation in a medium not containing the test substance for 72 hours. Further, "X->275->f" indicates treatment by another anticancer active substance in the initial treatment period of 24 hours, treatment by MS-275 in the following treatment period of 24 hours, then incubation in a medium not containing the test substance for 72 hours. Further, the numerical values showing the synergistic effect show the CI values.

Table 4

Synergistic Effect in Consecutive Combined Use of MS-275 and PTX

Cancer cell line		Time (hr)	Ratio 275:X	Order of consecutive combined use	
				275->X->f	X->275->f
Ovarian cancer	SK-OV-3	24+24+72	1000:1	1.1< -	0.76 ++
Breast cancer	T-47D	24+24+72	1000:1		0.71 ++

Table 5

Synergistic Effect in Consecutive Combined Use of MS-275 and GEM

Cancer cell line		Time (hr)	Ratio 275:X	Order of consecutive combined use	
				275->X->f	X->275->f
Colon cancer	HT-29	24+24+72	200:1	1.1< -	0.48 +++
Non-small cell lung cancer	NCI-H522	24+24+72	200:1	0.75 ++	1.1< -
	NCI-H522	24+24+72	3000:1		0.77 ++
	A549	24+24+72	100:1	1.1< -	0.69 +++
Ovarian cancer	OVCAR-3	24+24+72	400:1	1.1 -	0.54 +++
	SK-OV3	24+24+72	5000:1		0.56 +++
Pancreatic cancer	PANC-1	24+24+72	50000:1		0.59 +++

Table 6

Synergistic Effect in Consecutive Combined Use of MS-275 and CDDP

Cancer cell line		Time (hr)	Ratio 275:X	Order of consecutive combined use	
				275->X->f	X->275->f
Colon cancer	HCT116	24+24+72	1:8	0.63 +++	0.95 ±
	HT-29	24+24+72	4:1		0.89 +
Non-small cell lung cancer	NCI-H522	24+24+72	1:1	0.55 +++	0.69 +++
	A549	24+24+72	1:4	0.66 +++	0.42 +++
Ovarian cancer	SK-OV3	24+24+72	1:1	0.43 +++	0.57 +++
	OVCAR-3	24+24+72	1:1	0.77 ++	0.61 +++
Pancreatic cancer	PANC-1	24+24+72	8:1	0.96 ±	0.45 +++
	Capan-1	24+24+72	1:1	0.53 +++	0.63 +++

Table 7

Synergistic Effect in Consecutive Combined Use of MS-275 and CPT

Cancer cell line		Time (hr)	Ratio 275:X	Order of consecutive combined use	
				275->X->f	X->275->f
Colon cancer	HCT116	24+24+72	100:1	0.91 ±	0.85 ++
Non-small cell lung cancer	NCI-H522	24+24+72	100:1	0.31 +++	0.92 ±
	A549	24+24+72	25:1	1.1< -	0.79 ++
Ovarian cancer	OVCAR-3	24+24+72	200:1	1.05 ±	0.26 ++++
	SK-OV3	24+24+72	2000:1		0.72 ++
Pancreatic cancer	Capan-1	24+24+72	200:1	1.1< -	0.49 +++

Table 8

Synergistic Effect in Consecutive Combined Use of MS-275and DTX (Docetaxel)

Cancer cell line		Time (hr)	Ratio 275:X	Order of consecutive combined use	
				275->X->f	X->275->f
Non-small cell lung cancer	A549	24+24+72	10000:1		0.87 +
Ovarian cancer	SK-OV3	24+24+72	20000:1		0.87 +
Pancreatic cancer	Capan-1	24+24+72	3000:1		0.87 +
Prostate cancer	PC-3	24+24+72	300:1		0.89 +

Table 9

Synergistic Effect in Consecutive Combined Use of MS-275 Compound and CBDCA (Carboplatin)

Cancer cell line		Time (hr)	Ratio 275:X	Order of consecutive combined use	
				275->X->f	X->275->f
Non-small cell lung cancer	A549	24+24+72	1:10		0.31 +++
	NCI-H522	24+24+72	1:2		0.86 +
Ovarian cancer	SK-OV3	24+24+72	3:2		0.59 +++
Pancreatic cancer	Capan-1	24+24+72	1:1		0.47 +++
	PANC-1	24+24+72	1:1		0.30 ++++

Table 10

Synergistic Effect in Consecutive Combined Use of MS-275 and OXP (Oxaliplatin)

Cancer cell line		Time (hr)	Ratio 275:X	Order of consecutive combined use	
				275->X->f	X->275->f
Colon cancer	HT-29	24+24+72	5:1		0.77 ++
Ovarian cancer	SK-OV3	24+24+72	2:1		0.83 ++

Table 11

Synergistic Effect in Consecutive Combined Use of MS-275 and DOX (Doxorubicin)

Cancer cell line		Time (hr)	Ratio 275:X	Order of consecutive combined use	
				275->X->f	X->275->f
Ovarian cancer	SK-OV3	24+24+72	300:1		0.86 +

Table 12

Synergistic Effect in Consecutive Combined Use of MS-275 and VBL (Vinblastin)

Cancer cell line		Time (hr)	Ratio 275:X	Order of consecutive combined use	
				275->X->f	X->275->f
Non-small cell lung cancer	A549	24+24+72	300:1		0.89 +

Table 13

Synergistic Effect in Consecutive Combined Use of MS-275 and 5-FU (5-Fluorouracil)

Cancer cell line		Time (hr)	Ratio 275:X	Order of consecutive combined use	
				275->X->f	X->275->f
Colon cancer	HT-29	24+24+72	2:3		0.79 ++

In each case of each of the tested anticancer active substances, synergistic effects due to combined use with MS-275 were detected.

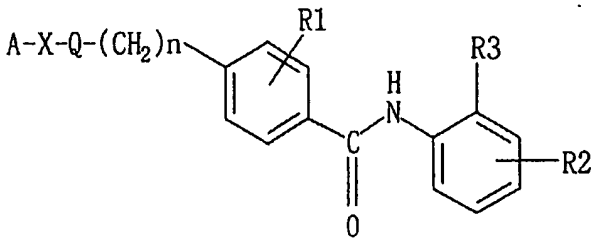
INDUSTRIAL APPLICABILITY

As explained above, synergistic effects are recognized in in vitro tests between histone deacetylase inhibitors as represented by MS-275 and other various types of known anticancer active substances, so it is suggested that synergistic effects will be obtained in treatment for human cancer patient as well.

CLAIMS

1. A pharmaceutical composition or a combination comprising, as active ingredients:

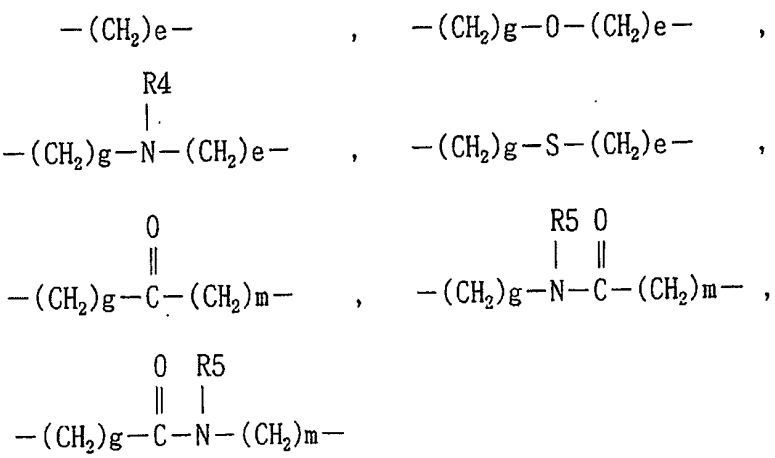
(a) at least one of the benzamide derivatives which is a histone deacetylase inhibiting substance, or a pharmaceutically acceptable salt thereof, represented by the following formula (1):



(1)

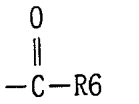
wherein A is an optionally substituted phenyl group or an optionally substituted heterocyclic group wherein the substituent(s) for the phenyl group or the heterocyclic group is (are) 1 to 4 substituents selected from the group consisting of a halogen atom, a hydroxyl group, an amino group, a nitro group, a cyano group, an alkyl group having 1 to 4 carbons, an alkoxy group having 1 to 4 carbons, an aminoalkyl group having 1 to 4 carbons, an alkylamino group having 1 to 4 carbons, an acyl group having 1 to 4 carbons, an acylamino group having 1 to 4 carbons, an alkylthio group having 1 to 4 carbons, a perfluoroalkyl group having 1 to 4 carbons, a perfluoroalkyloxy group having 1 to 4 carbons, a carboxyl group, an alkoxycarbonyl group having 1 to 4 carbons, a phenyl group and a heterocyclic group;

X is a bond or a moiety having a structure selected from those illustrated in formula (2):



(2)

wherein e is an integer of 1 to 4; g and m are independently an integer of 0 to 4; R4 is a hydrogen atom or an optionally substituted alkyl group having 1 to 4 carbons, or the acyl group represented by formula (3)

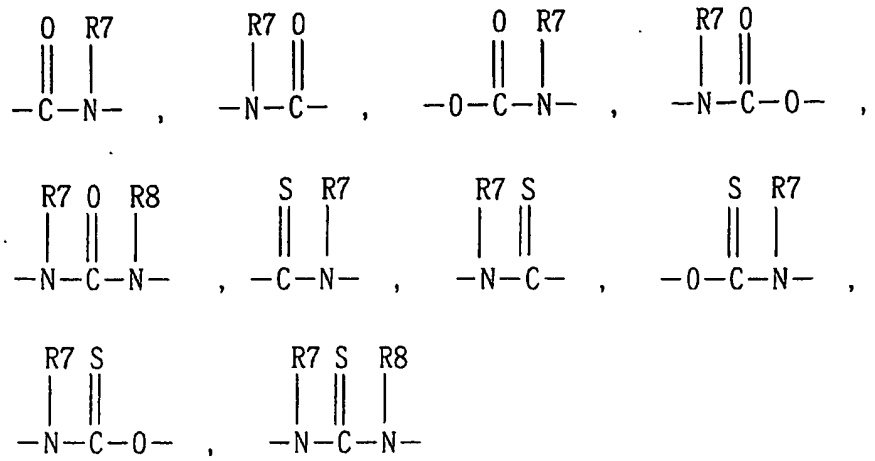


(3)

wherein R6 is an optionally substituted alkyl group having 1 to 4 carbons, a perfluoroalkyl group having 1 to 4 carbons, a phenyl group or a heterocyclic group; R5 is a hydrogen atom or an optionally substituted alkyl group having 1 to 4 carbons;

n is an integer of 0 to 4, provided that when X is a bond, n is not zero;

Q is a moiety having a structure selected from those illustrated in formula (4)



(4)

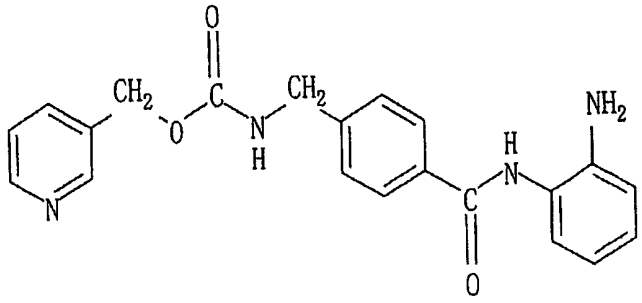
wherein R7 and R8 are independently a hydrogen atom or an optionally substituted alkyl group having 1 to 4 carbons;

R1 and R2 are independently a hydrogen atom, a halogen atom, a hydroxyl group, an amino group, an alkyl group having 1 to 4 carbons, an alkoxy group having 1 to 4 carbons, an aminoalkyl group having 1 to 4 carbons, an alkylamino group having 1 to 4 carbons, an acyl group having 1 to 4 carbons, an acylamino group having 1 to 4 carbons, an alkylthio group having 1 to 4 carbons, a perfluoroalkyl group having 1 to 4 carbons, a perfluoroalkyloxy group having 1 to 4 carbons, a carboxyl group or an alkoxycarbonyl group having 1 to 4 carbons;

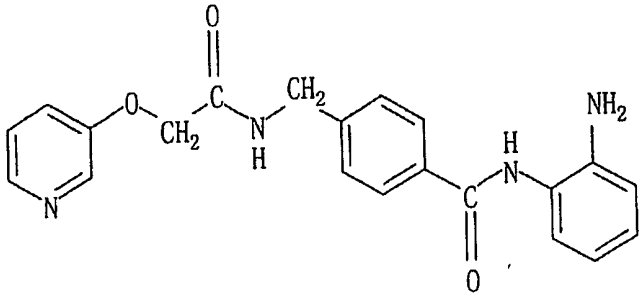
R3 is a hydroxyl group or amino group, and

(b) at least one of the substances which is another anti-cancer active substance selected from a group consisting of cisplatin, etoposide, camptothecin, 5-fluorouracil, gemcitabine, paclitaxel, docetaxel, carboplatin, oxaliplatin, doxorubicin and vinblastin.

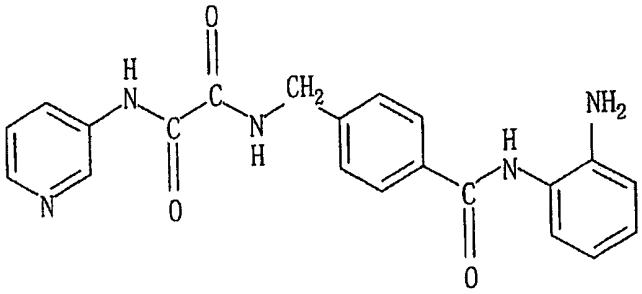
2. A pharmaceutical composition or a combination according to claim 1 wherein said benzamide derivative is selected from formulas (5) to (8) or a pharmaceutically acceptable salt thereof.



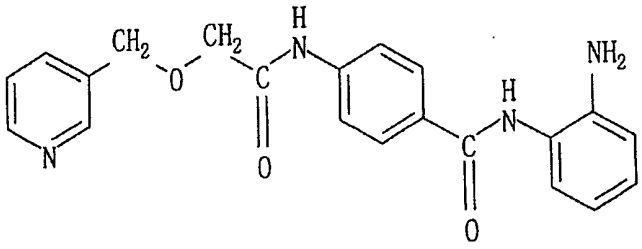
(5)



(6)

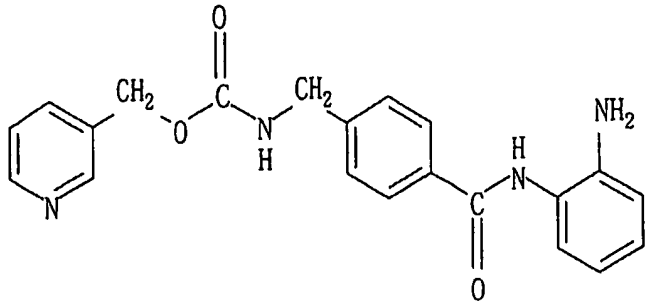


(7)



(8)

3. A pharmaceutical composition or a combination according to claim 1 or 2 wherein said benzamide derivative is represented by formula (5) or a pharmaceutically acceptable salt thereof.



(5)

4. A pharmaceutical composition or a combination according to any one of claims 1 to 3 wherein a substance selected from a group of substances consisting of said ingredient (b) which is another anti-cancer active substance is cisplatin.

5. A pharmaceutical composition or a combination according to claim 4, which is used for treatment of non-small cell lung cancer, ovarian cancer, colon cancer or pancreatic cancer.

6. A pharmaceutical composition or a combination according to any one of claims 1 to 3 wherein a substance selected from a group of substances consisting of said ingredient (b) which is another anti-cancer active substance is etoposide.

7. A pharmaceutical composition or a combination according to claim 6, which is used for treatment of ovarian cancer.

8. A pharmaceutical composition or a combination according to any one of claims 1 to 3 wherein a substance selected from a group of substances consisting of said ingredient (b) which is another anti-cancer active substance is camptothecin.

9. A pharmaceutical composition or a combination according to claim 8, which is used for treatment of non-small cell lung cancer, ovarian cancer, colon cancer or pancreatic cancer.

10. A pharmaceutical composition or a combination according to any one of claims 1 to 3 wherein a substance selected from a group of substances consisting of said

ingredient (b) which is another anti-cancer active substance is 5-fluorouracil.

11. A pharmaceutical composition or a combination according to claim 10, which is used for treatment of breast cancer or colon cancer.

12. A pharmaceutical composition or a combination according to any one of claims 1 to 3 wherein a substance selected from a group of substances consisting of said ingredient (b) which is another anti-cancer active substance is gemcitabine.

13. A pharmaceutical composition or a combination according to claim 12, which is used for treatment of non-small cell lung cancer, ovarian cancer, colon cancer or pancreatic cancer.

14. A pharmaceutical composition or a combination according to any one of claims 1 to 3 wherein a substance selected from a group of substances consisting of said ingredient (b) which is another anti-cancer active substance is paclitaxel.

15. A pharmaceutical composition or a combination according to claim 14, which is used for treatment of breast cancer, ovarian cancer or prostate cancer.

16. A pharmaceutical composition or a combination according to any one of claims 1 to 3 wherein a substance selected from a group of substances consisting of said ingredient (b) which is another anti-cancer active substance is docetaxel.

17. A pharmaceutical composition or a combination according to claim 16, which is used for treatment of non-small cell lung cancers, ovarian cancer, pancreatic cancer and prostate cancer.

18. A pharmaceutical composition or a combination according to any one of claims 1 to 3 wherein a substance selected from a group of substances consisting of said ingredient (b) which is another anti-cancer active substance is carboplatin.

19. A pharmaceutical composition or a combination

according to claim 18, which is used for treatment of non-small cell lung cancer, ovarian cancer, or pancreatic cancer.

20. A pharmaceutical composition or a combination according to any one of claims 1 to 3 wherein a substance selected from a group of substances consisting of said ingredient (b) which is another anti-cancer active substance is oxaliplatin.

21. A pharmaceutical composition or a combination according to claim 20, which is used for treatment of colon cancer or ovarian cancer.

22. A pharmaceutical composition or a combination according to any one of claims 1 to 3 wherein a substance selected from a group of substances consisting of said ingredient (b) which is another anti-cancer active substance is doxorubicin.

23. A pharmaceutical composition or a combination according to claim 22, which is used for treatment of ovarian cancer.

24. A pharmaceutical composition or a combination according to any one of claims 1 to 3 wherein a substance selected from a group of substances consisting of said ingredient (b) which is another anti-cancer active substance is vinblastin.

25. A pharmaceutical composition or a combination according to claim 24, which is used for treatment of non-small cell lung cancer.

26. A pharmaceutical combination according to any one of claims 1 to 25, of which said ingredient (a) which is a histone deacetylase inhibiting substance and said ingredient (b) which is another anti-cancer active substance are sequentially administered to patients.

27. A pharmaceutical combination according to claim 26, wherein said ingredient (b) which is another anti-cancer active substance is paclitaxel.

28. A pharmaceutical combination according to claim 27, of which the administration sequence is paclitaxel

and then said ingredient (a) which is a histone deacetylase inhibiting substance.

29. A pharmaceutical combination according to claim 28, which is used for treatment of ovarian cancer or breast cancer.

30. A pharmaceutical combination according to claim 26, wherein said ingredient (b) which is another anti-cancer active substance is cisplatin.

31. A pharmaceutical combination according to claim 30, of which the administration sequence is said ingredient (a) which is a histone deacetylase inhibiting substance and then cisplatin.

32. A pharmaceutical combination according to claim 31, which is used for treatment of non-small cell lung cancer, ovarian cancer, colon cancer or pancreatic cancer.

33. A pharmaceutical combination according to claim 30, of which the administration sequence is cisplatin and then said ingredient (a) which is a histone deacetylase inhibiting substance.

34. A pharmaceutical combination according to claim 33, which is used for treatment of non-small cell lung cancer, ovarian cancer, colon cancer or pancreatic cancer.

35. A pharmaceutical combination according to claim 26, wherein said ingredient (b) which is another anti-cancer active substance is camptothecin.

36. A pharmaceutical combination according to claim 35, of which the administration sequence is said ingredient (a) which is a histone deacetylase inhibiting substance and then camptothecin.

37. A pharmaceutical combination according to claim 36, which is used for treatment of non-small cell lung cancer.

38. A pharmaceutical combination according to claim 35, of which the administration sequence is camptothecin and then said ingredient (a) which is a histone

deacetylase inhibiting substance.

39. A pharmaceutical combination according to claim 38, which is used for treatment of non-small cell lung cancer, ovarian cancer, colon cancer or pancreatic cancer.

40. A pharmaceutical combination according to claim 26, wherein said ingredient (b) which is another anti-cancer active substance is gemcitabine.

41. A pharmaceutical combination according to claim 40, of which the administration sequence is said ingredient (a) which is a histone deacetylase inhibiting substance and then gemcitabine.

42. A pharmaceutical combination according to claim 41, which is used for treatment of non-small cell lung cancer.

43. A pharmaceutical combination according to claim 40, of which the administration sequence is gemcitabine and then said ingredient (a) which is a histone deacetylase inhibiting substance.

44. A pharmaceutical combination according to claim 43, which is used for treatment of non-small cell lung cancer, ovarian cancer, pancreatic cancer or colon cancer.

45. A pharmaceutical combination according to claim 26, wherein said ingredient (b) which is another anti-cancer active substance is 5-fluorouracil.

46. A pharmaceutical combination according to claim 45, of which the administration sequence is 5-fluorouracil and then said ingredient (a) which is a histone deacetylase inhibiting substance.

47. A pharmaceutical combination according to claim 46 which is used for treatment of colon cancer.

48. A pharmaceutical combination according to claim 26, wherein said ingredient (b) which is another anti-cancer active substance is docetaxel.

49. A pharmaceutical combination according to claim 48, of which the administration sequence is docetaxel and

then said ingredient (a) which is a histone deacetylase inhibiting substance.

50. A pharmaceutical combination according to claim 49 which is used for treatment of non-small cell lung cancer, ovarian cancer, pancreatic cancer or prostate cancer.

51. A pharmaceutical combination according to claim 26, wherein said ingredient (b) which is another anti-cancer active substance is carboplatin.

52. A pharmaceutical combination according to claim 51, of which the administration sequence is carboplatin and then said ingredient (a) which is a histone deacetylase inhibiting substance.

53. A pharmaceutical combination according to claim 52 which is used for treatment of non-small cell lung cancer, ovarian cancer or pancreatic cancer.

54. A pharmaceutical combination according to claim 26, wherein said ingredient (b) which is another anti-cancer active substance is oxaliplatin.

55. A pharmaceutical combination according to claim 54, of which the administration sequence is oxaliplatin and then said ingredient (a) which is a histone deacetylase inhibiting substance.

56. A pharmaceutical combination according to claim 55 which is used for treatment of colon cancer or ovarian cancer.

57. A pharmaceutical combination according to claim 26, wherein said ingredient (b) which is another anti-cancer active substance is doxorubicin.

58. A pharmaceutical combination according to claim 57, of which the administration sequence is doxorubicin and then said ingredient (a) which is a histone deacetylase inhibiting substance.

59. A pharmaceutical combination according to claim 58 which is used for treatment of ovarian cancer.

60. A pharmaceutical combination according to claim 26, wherein said ingredient (b) which is another anti-

cancer active substance is vinblastin.

61. A pharmaceutical combination according to claim 60, of which the administration sequence is vinblastin and then said ingredient (a) which is a histone deacetylase inhibiting substance.

62. A pharmaceutical combination according to claim 61 which is used for treatment of non-small cell lung cancer.

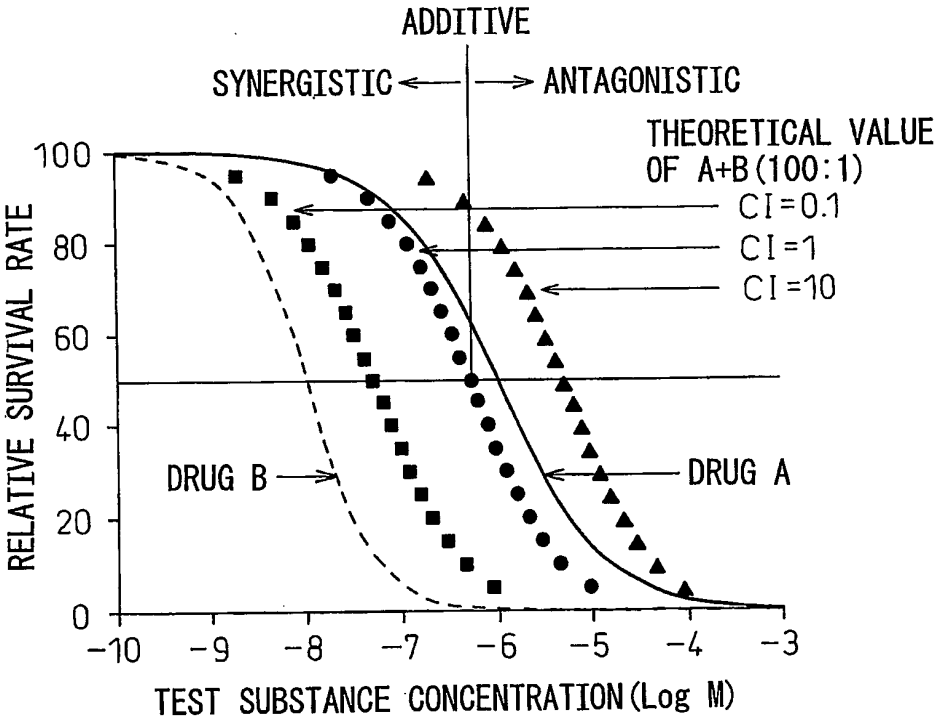
63. A cancer treatment kit comprising a pharmaceutical combination according to any one of claims 1 - 62, which comprises:

(i) at least one of said ingredients (a) which is a histone deacetylase inhibiting substance,

(ii) at least one of said ingredients (b) which is another anti-cancer active substance, and

(iii) an instruction for administration schedule for simultaneous or sequential administration according to a kind of cancer (for sequential administration to a patient at periodic intervals).

Fig.1


ADDITIVE
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TEST SUBSTANCE CONCENTRATION (Log M)

INTERNATIONAL SEARCH REPORT

International Application No
rCT/JP2004/007562

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/4406 A61K33/24 A61K31/282 A61K31/70 A61K31/4745
A61K31/513 A61K31/7068 A61K31/337 A61K31/555 A61K31/704
A61P35/00 A61K31/475

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 273 296 A (WARNER LAMBERT CO) 8 January 2003 (2003-01-08) paragraphs '0003!, '0004!, '0011! - '0013!, '0032!; claims	1,16,17, 26, 48-50,63
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 August 2004	02/09/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Paul Soto, R

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INTERNATIONAL SEARCH REPORT

International Application No
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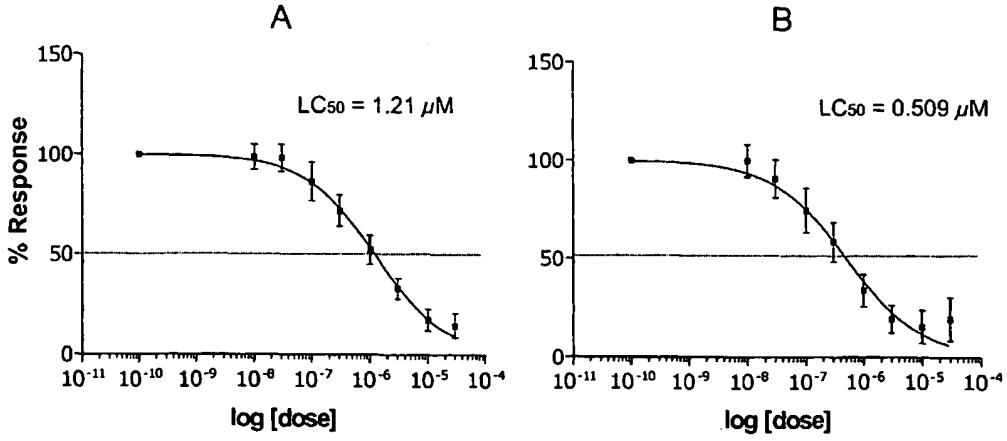
(54) Title: TREATMENT OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA



(57) Abstract: The invention provides methods for increasing expression of 1D10 antigen in B-CLL cells using the benzamide derivatives. The invention provides methods for treating a patient with chronic lymphocytic leukemia of B cells (B-CLL) are also provided. One method comprises administering to a patient with B-CLL, one or more benzamide derivatives, the benzamide derivatives including MS-275 and related compounds. Another method comprises administering to a patient with B-CLL, one or more of the benzamide derivatives, and additionally administering to the patient one or more antibodies immunospecific for 1D10 antigen.

WO 2005/007158 A1

TREATMENT OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

This application claims priority from U.S. Provisional Patent Application Serial Number 60/368,775, filed on March 29, 2002, which is incorporated herein by reference. This invention was made, at least in part, with government support under National Institutes of Health Grant 2P01 CA81534. The U.S. government has certain rights in the invention.

Field of the Invention

This invention relates to methods for treating a patient with B cell chronic lymphocytic leukemia (B-CLL) by administering agents comprising benzamide derivatives such as MS-275, and optionally, additionally administering antibodies immunospecific for the 1D10 antigen to the patient.

Background

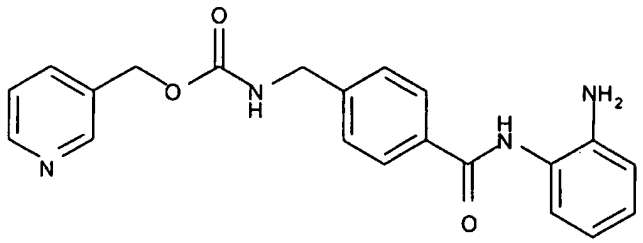
Chronic lymphocytic leukemia (CLL) of B cells (B-CLL) is the most common type of adult leukemia. One in four human leukemias are of this type. In B-CLL, B cells are halted in their normal differentiation process and, therefore, are incapable of performing a variety of normal functions, and are resistant to normal programmed cell death. These B cells, herein called B-CLL cells, are malignant and accumulate in the blood of patients during the disease. Although the cells are not found to be undergoing DNA synthesis or mitosis, they accumulate in the blood of the B-CLL patient over a long period of time, often years. Accumulation of these inactive, non-proliferating B-CLL cells interferes with normal immune function in the patient, resulting in potentially fatal cytopenias and infections.

Accepted treatment regimens for patients with B-CLL consist of administration of a variety of therapeutic anti-CLL agents, including nucleoside analogs or alkylating agents, and current trials are investigating the benefits of combinations of these agents with monoclonal antibodies. However, therapeutic options for patients with B-CLL are limited, and in most cases, are ineffective or have a limited period of effectiveness. Relapse of the disease often occurs and these patients acquire resistance, not only to the drug used for patient treatment, but to other drugs as well.

Given these factors, discovery of new therapies and therapeutic combinations is critical in order to make significant progress in improving overall patient condition and survival in this disease.

Summary of the Invention

The present invention provides methods for treating a patient suspected of having or known to have chronic lymphocytic leukemia (CLL) of B cells (B-CLL). One method comprises administering to a patient, a biologically effective amount of one or more compounds of the structures as pictured in I, II or III below:



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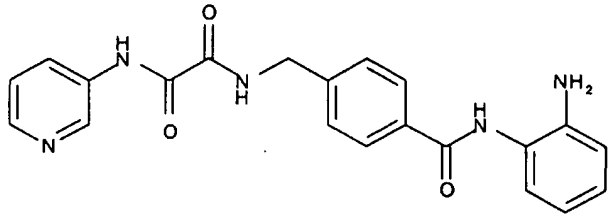

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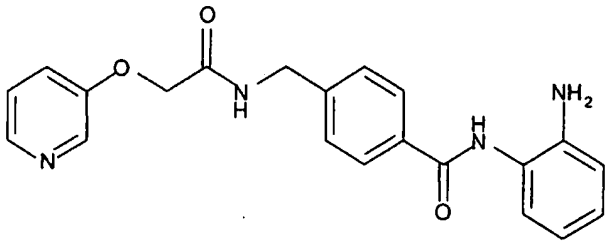


(III)

Another method comprises administering to a patient a biologically effective amount of one or more of the compounds of the structures as pictured in I, II or III above, and administering a biologically effective amount of one or more antibodies immunospecific for 1D10 antigen. The invention also provides methods for inducing apoptosis of B-CLL cells by contacting the cells with the compounds of the structures as pictured in I, II or III above. The invention also provides methods for inducing or increasing expression of 1D10 antigen on B-CLL cells by contacting the cells with the compounds of the structures as pictured in I, II or III above.

Brief Description of the Drawings

Figure 1. LC_{50} determination of MS-275 in primary B-CLL cells. Peripheral blood mononuclear cells (PBMCs) from thirteen individual B-CLL patients were isolated and incubated

with varying concentrations of MS-275 from 0.01 to 100 uM. **A**. Cells were incubated in drug for 4 hours and were then washed and re-incubated in fresh media for a total of 96 hours. **B**. Cells were incubated in drug for 96 hours continuously. Viability was determined by an MTT assay to detect metabolic activity. Each patient sample at each concentration was run in quadruplicate and was normalized to cells from the same patient incubated for the same time period in media alone. Error bars represent 95% confidence intervals.

Figure 2. MS-275 induced acetylation of histone proteins. PBMCs from two different B-CLL patients (Patient No. 1 and 2) were collected and incubated with 0, 0.3, or 3.0 μM MS-275 for 24 hours. Nuclear extracts were prepared from the cells and were separated by SDS-polyacrylamide gel electrophoresis before immunoblotting with antibodies immunospecific for acetylated forms of H3 or H4 histones. Coomassie blue staining was used to control for protein amount loaded onto the SDS polyacrylamide gels.

Figure 3. MS-275 treatment of patient B-CLL cells resulted in apoptosis. B-CLL patient cells were incubated with 0, 0.3, or 3.0 μM MS-275 for 24 hours. Apoptosis was assessed by annexin V/propidium iodide flow cytometry.

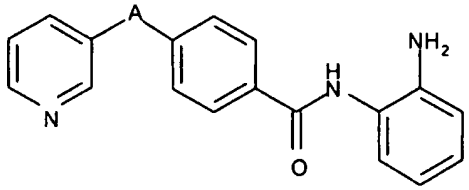
Figure 4. MS-275 treatment of patient B-CLL cells resulted in cleavage of caspase 3. B-CLL patient cells were incubated with 0.3 or 3.0 μM MS-275 for 24 hours. At these time points, lysates were prepared from the cells and caspase 3 activity in the lysates was measured using a colorimetric assay.

Detailed Description of the Invention

The malignant cells from patients with B cell chronic lymphocytic leukemia (B-CLL) are herein called B-CLL cells. These malignant B-CLL cells are halted in their normal differentiation process and are not found to be undergoing DNA synthesis or mitosis. These cells express relatively low levels of 1D10 antigen.

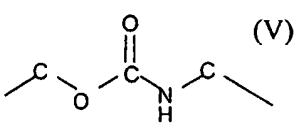
We have found that the compound MS-275, also called MS-27-275 or N-(2-aminophenyl)-4-[N-(pyridin-3-yl-methoxycarbonyl) aminomethyl] benzamide, is selectively toxic to B-CLL cells from patients with B-CLL compared to cells in peripheral blood mononuclear cell (PBMC) preparations from individuals who do not have B-CLL (normal individuals). We have found that the selective toxicity of MS-275 for B-CLL cells is due, at least in part, to induction of apoptosis of the B-CLL cells. We have also found that MS-275 induces increased expression of 1D10 antigen on the surface of B-CLL cells. Therapeutic antibodies exist that specifically bind to or are immunospecific for the 1D10 antigen.

Based on these findings, the present invention provides for methods of treating a patient who has B-CLL. In one embodiment, the method comprises administering a biologically effective amount of one or more benzamide derivatives to a patient that comprise the general chemical structure below, shown as IV:

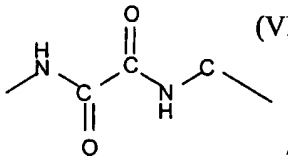


(IV)

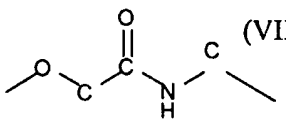
wherein A represents a structure shown by either of the formulas V, VI or VII:



(V)



(VI)



(VII)

In another embodiment, the method comprises administering a biologically effective amount of one or more of the above-described benzamide derivatives to a patient and administering a biologically effective amount of one or more antibodies immunospecific for 1D10 antigen to the patient.

MS-275 and Related Compounds

MS-275 is a synthetic benzamide derivative that has been shown to inhibit cellular histone deacetylase activity and to block growth in a variety of human tumor cell lines (A. Saito, et al., 1999, Proc. Natl. Acad. Sci. USA, A synthetic inhibitor of histone deacetylase, MS-27-275, with marked *in vivo* antitumor activity against human tumors, 96:4592-7). The chemical structure of MS-275 is shown as structure I in the Summary of the Invention section of this application. MS-275 is chemically synthesized using methods known in the art. One such method is described in T. Suzuki et al., 1999, J. Med. Chem., Synthesis and histone deacetylase inhibitory activity of new benzamide derivatives, 42:3001-3. Additional information relating to synthesis of MS-275 and related compounds is found in Japanese Unexamined Patent Publication Hei No. 10-152462. MS-275 is also available from various sources. One such source is Nihon Schering K.K. Another source is the National Cancer Institute (MS-275 is NSC No. 706995).

Two benzamide derivatives closely related to MS-275 are shown as structures II and III in the Summary of the Invention section of this application.

In addition to the benzamide derivative compounds shown as structures I, II and III in the Summary of the Invention section of this application, pharmaceutically acceptable salts of the benzamide derivatives may be used in practice of the invention. Such salts include salts with an inorganic acid such as hydrochloric acid, hydrobromic acid, sulfuric acid and phosphoric acid; and with an organic acid such as acetic acid, lactic acid, tartaric acid, malic acid, succinic acid, fumaric acid, maleic acid, citric acid, benzoic acid, trifluroacetic acid, p-toluenesulfonic acid and methanesulfonic acid. Such salts include N-(2-aminophenyl)-4-(N-(pyridin-3-yl)methoxycarbonylaminomethyl)benzamide hydrochloride, N-(2-aminophenyl)-4-(N-(pyridin-3-yl)methoxycarbonylaminomethyl)benzamidehydrobromide, N-(2-aminophenyl)-4-(N-(pyridin-3-yl)methoxycarbonylaminomethyl)benzamide sulfate, N-(2-aminophenyl)-4-(N-(pyridin-3-yl)methoxycarbonylaminomethyl)benzamide phosphate, N-(2-aminophenyl)-4-(N-(pyridin-3-yl)methoxycarbonylaminomethyl)benzamide acetate, N-(2-aminophenyl)-4-(N-(pyridin-3-yl)methoxycarbonylaminomethyl)benzamide lactate, N-(2-aminophenyl)-4-(N-(pyridin-3-yl)methoxycarbonylaminomethyl)benzamide tartrate, N-(2-aminophenyl)-4-(N-(pyridin-3-yl)methoxycarbonylaminomethyl)benzamide malate, N-(2-aminophenyl)-4-(N-(pyridin-3-yl)methoxycarbonylaminomethyl)benzamide succinate, N-(2-aminophenyl)-4-(N-(pyridin-3-yl)methoxycarbonylaminomethyl)benzamide fumarate, N-(2-aminophenyl)-4-(N-(pyridin-3-yl)methoxycarbonylaminomethyl)benzamide maleate, N-(2-aminophenyl)-4-(N-(pyridin-3-yl)methoxycarbonylaminomethyl)benzamide citrate, N-(2-aminophenyl)-4-(N-(pyridin-3-yl)methoxycarbonylaminomethyl)benzamide trifluoroacetate, N-(2-aminophenyl)-4-(N-(pyridin-3-yl)methoxycarbonylaminomethyl)benzamide p-toluenesulfonate and N-(2-aminophenyl)-4-(N-(pyridin-3-yl)methoxycarbonylaminomethyl)benzamide methanesulfonate, and others.

1D10 Antigen and Antibodies

The 1D10 antigen was originally identified as an epitope on the surface of malignant B lymphocytes (R.D. Gingrich et al., 1990, Identification and characterization of a new surface membrane antigen found predominantly on malignant B lymphocytes, Blood 75:2375-87).

Antibodies immunospecific for the 1D10 antigen have been developed (see for example, U.S. Pat No. 6,129,914 to Weiner et al., issued October 10, 2000) and can induce complement-mediated cytotoxicity, antibody-dependent cell cytotoxicity and/or apoptosis of cells expressing the 1D10 antigen (see for example, S.A. Kostelny et al., 2001, Humanization and characterization of the anti-HLA-DR antibody 1D10, Int. J. Cancer 93:556-65).

Various forms of an antibody immunospecific for 1D10 antigen may be used in practice of the methods of this invention. For example, the 1D10 antibody may be a full length antibody (e.g., having a human immunoglobulin constant region) or an antibody fragment (e.g. a $F(ab')_2$). The term "antibody" as used herein encompasses monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity of binding to 1D10 antigen. "Antibody fragments" comprise a portion of a full length antibody, generally the antigen binding or variable region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by G. Kohler & C. Milstein, 1975, Nature 256:495-7, or may be made by recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567 to Cabilly et al., issued March 28, 1989). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson et al., 1991, Nature 352:624-8 and Marks et al., 1991, J. Mol. Biol. 222:581-97, for example.

The monoclonal antibodies herein include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity.

"Single-chain Fv" or "sFv" antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the sFv to form the desired structure for antigen binding.

An "isolated" antibody is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-polyacrylamide gel electrophoresis under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step

In order to avoid potential immunogenicity of the monoclonal antibodies in human, the monoclonal antibodies that have the desired function are preferably humanized. "Humanized" forms of non-human (e.g., murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., 1986, Nature 321:522-5, Reichmann et al., 1988, Nature 332:323-9, and Presta, 1992, Curr. Op. Struct. Biol. 2:593-6.

Methods of making an 1D10 antibody, particularly a humanized form of the 1D10 antibody, are described in U.S. Patent No. 6,129,914 to Weiner et al,. issued October 10, 2000, the description of which is specifically incorporated herein by reference. Such antibodies are also available from, for example, Protein Design Labs, Inc., Fremont, CA.

The preferred portion of the 1D10 antigen on B-CLL cells to which the 1D10 monoclonal antibody binds, or is immunospecific for, is a heterodimeric polypeptide which contains two proteins with a molecular weight of the alpha and beta chains being 32 kDa and 28 kDa, respectively. The proteins can be obtained by solubilizing cells expressing the antigen with detergent. Molecular weight determination is made by using iodinated cells and single dimension SDS-polyacrylamide gel electrophoresis analysis of an antibody precipitate obtained from extracts of the iodinated cells. The formation of one 1D10 antibody is discussed by Gingrich et al., 1990, Blood 75:2375-2387. Other antibodies having the same or similar binding specificity to 1D10 are screened by competition binding with 1D10 to the 28/32 kDa heterodimeric antigen. Numerous types of competitive binding assays are known, for example: solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), sandwich competition assay (see Stahli et al., 1983, Methods in Enzymology 9:242-53), solid phase direct biotin-avidin EIA (see Kirkland et al., 1986, J. Immunol. 137:3614-9), solid phase direct labeled assay, solid phase direct labeled sandwich assay (see Harlow & Lane, 1988, "Antibodies, A Laboratory Manual," Cold Spring Harbor Press); solid phase direct label RIA using I-125 label (see Morel et al., 1988, Molec. Immunol. 25:7-15), solid phase direct biotin-avidin EIA (Cheung et al., 1990, Virology 176:546-52); and direct labeled RIA (Moldenhauer et al., 1990, Scand. J. Immunol. 32:77-82). Typically, such an assay involves the use of cells bearing the 28/32 kDa antigen, an unlabelled test immunoglobulin and a labeled reference immunoglobulin (1D10). Competitive inhibition is measured by determining the amount of label bound to the cells in the presence of the test immunoglobulin. Usually the test immunoglobulin is present in excess. Antibodies identified by competition assay (competing antibodies) include antibodies binding to the same epitope as the reference antibody and antibodies binding to an adjacent epitope sufficiently proximal to the epitope bound by the reference antibody for steric hindrance to occur.

Pharmaceutical Compositions

The benzamide derivatives shown as structures I, II or III in the Summary of the Invention section of this application are preferably parts of pharmaceutical compositions intended for administration to a patient.

The benzamide derivatives, or the pharmaceutically acceptable salts thereof, may be prepared with generally used diluents, excipients, vehicles and additives such as filler, extender, binder, carrier, salt, moisturizing agent, disintegrator, disintegrator retarder, absorption promoters, adsorbent, glidant, buffering agent, preservative, dispersing agent, wetting agent,

suspending agent, surfactant, lubricant and others. The benzamide derivatives or the pharmaceutically acceptable salts thereof may have a variety of dosage forms depending on their therapeutic purpose; typically tablet, pill, powder, solution, suspension, emulsion, granule, capsule, injection (e.g., solution, suspension) and suppository.

For preparing tablets, a variety of carriers well-known in the art may be used. Such a carrier includes excipients such as lactose, glucose, starch, calcium carbonate, hydrogenated vegetable oil, kaoline, crystalline cellulose and silicic acid; binders such as water, ethanol, propanol, simple syrup, glucose solution, starch solution, gelatin solution, carboxymethyl cellulose, shellac, methyl cellulose and polyvinyl pyrrolidone, powdered acacia, powdered tragacanth gum and gelatin; disintegrators such as calcium carmelose, agar, dried starch, sodium alginate, powdered agar, calcium carmelose, starch and lactose; disintegration retarders such as sucrose, cocoa butter and hydrogenated oil; absorption promoters such as quaternary ammonium base and sodium lauryl sulfate; moisturizing agents such as glycerin and starch; adsorbents such as starch, lactose, kaoline, bentonite, colloidal silicic acid; and glidants such as talc, stearates and polyethylene glycol. The tablet may be, if necessary, one coated with a common coating; for example, sugar-coated tablet, gelatin-coated tablet, enteric coated tablet, film-coated tablet, double-layer tablet and multilayer tablet.

Capsules may be prepared by blending an active ingredient with a variety of the above carriers as usual and filling the resulting blend into, for example, a hard or soft gelatin capsule or the like.

Injection, solution, emulsion and suspension forms of the benzamide derivatives or their pharmaceutically acceptable salts are sterilized and preferably isotonic with blood. Such forms may be prepared using diluents commonly used in the art; for example, water, ethanol, macrogol, propylene glycol, ethoxylated isostearyl alcohol, polyoxyisostearyl alcohol and polyoxyethylene sorbitan fatty acid esters. The pharmaceutical preparation may contain sodium chloride necessary to prepare an isotonic solution, glucose or glycerin, as well as usual solubilizers, buffers and soothing agents.

Compositions suitable for parenteral administration conveniently comprise a sterile, pyrogen-free, aqueous or oleaginous preparation of the benzamide derivatives which are preferably isotonic with the blood of the recipient. This aqueous preparation may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation also may be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution,

and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. In addition, fatty acids such as oleic acid may be used in the preparation of injectables. Carrier formulations suitable for oral, subcutaneous, intravenous, intramuscular, etc. administrations can be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA. The pharmaceutical compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well-known in the art of pharmacy.

Additionally, preparation of parenterally-acceptable solutions of the pharmaceutical composition, having due regard to pH, isotonicity, stability, and the like, is within the level of ordinary skill in the art of pharmacy and pharmacology. A preferred pharmaceutical composition for injection can contain, in addition to the vector, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, phosphate buffered saline (PBS), or other vehicle as known in the art. The pharmaceutical composition used in the methods of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

Suppository forms of the benzamide derivatives or their pharmaceutically acceptable salts may be prepared using a variety of well-known carriers; for example, semi-synthetic glyceride, cocoa butter, higher alcohols, higher alcohol esters and polyethylene glycol.

Furthermore, the composition may contain coloring agents, preservatives, perfumes, flavors, sweeteners and/or other drugs. The amount of the active ingredient in the composition may be, as appropriate, selected from a wide range with no limitations, and is generally about 1 to 70% by weight in the composition, preferably about 5 to 50% by weight.

The antibodies immunospecific for 1D10 are preferably parts of pharmaceutical compositions intended for administration to a patient. Pharmaceutical compositions comprising antibodies of the present invention are useful for parenteral administration, i.e., subcutaneously, intramuscularly and particularly, intravenously. The compositions for parenteral administration commonly comprise a solution of the antibody or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine and the like. These solutions are sterile and generally free of particulate matter. The compositions may contain pharmaceutically-acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example sodium acetate, sodium

chloride, potassium chloride, calcium chloride and sodium lactate. The concentration of the antibodies in these formulations can vary widely, i.e., from less than about 0.01%, usually at least about 0.1% to as much as 5% by weight and will be selected primarily based on fluid volumes and viscosities in accordance with the particular mode of administration selected. A typical composition for intravenous infusion can be made up to contain 250 ml of sterile Ringer's solution, and 10 mg of antibody (see Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA).

Administration of the Pharmaceutical Compositions

An administration route of the benzamide derivatives or pharmaceutically acceptable salt thereof is not limited, and is selected depending on patient's age, sex, severity of disease and other conditions. For example, tablet, pill, solution, suspension, emulsion, granule and capsule may be orally administered (i.e., through the mouth); injection may be intravenously administered solely or in combination with a common infusion fluid such as glucose, amino acids and the like, or if necessary, intramuscularly, subcutaneously or intraperitoneally as a sole preparation. Suppository may be intrarectally administered. Advantageously, the benzamide agent or its pharmaceutically acceptable salt, may be administered orally.

A "biologically effective amount" as used herein in reference to the benzamide derivatives, means an amount of the benzamide derivative sufficient to decrease the viability of B-CLL cells from patients with B-CLL, induce apoptosis of B-CLL cells from patients with B-CLL or induce or increase expression of 1D10 antigen in B-CLL cells from patients with B-CLL. Preferably, biologically effective amounts of the benzamide derivatives ameliorate the pathological effects of B-CLL in patients with the disease. Ultimately, the dosage will be determined using clinical trials. Initially, the clinician will administer doses that have been derived from animal studies. The effective amount can be achieved by one administration of the composition. Alternatively, the effective amount is achieved by multiple administrations of the composition to the animal.

Dose of the benzamide derivative may be selected, depending on their dosage form, patient's age, sex and severity of disease, and other conditions, as appropriate, but the amount of the active ingredient may be generally about 0.0001 to 100 mg/kg a day. It is recommended that a unit dosage form may contain about 0.001 to 1000 mg of the active ingredient.

The invention further provides for administration of the benzamide derivatives and a pharmaceutically effective amount of one or more 1D10 antibodies, preferably humanized anti-1D10 monoclonal antibodies, to a patient suspected of or known to have B-CLL. While it is

possible to administer these two agents in any order or simultaneously, it is preferred that the benzamide derivative is administered before the antibody.

The compositions containing the antibodies immunospecific for 1D10 antigen are generally administered therapeutically, meaning the compositions are administered to a patient known to have or suspected of having B-CLL. The compositions containing the antibodies are administered in biologically effective amounts, meaning an amount sufficient to completely or partially arrest the B-CLL disease and its complications. Amounts effective for this use will depend upon the severity of the condition and the general state of the patient's own immune system, but generally range from about 0.01 to about 100 mg of antibody per dose, with dosages of from 0.1 to 50 mg and 1 to 10 mg per patient being more commonly used. Single or multiple administrations on a daily, weekly or monthly schedule can be carried out with dose levels and pattern being selected by the treating physician.

In some methods of treatment, the benzamide derivatives or benzamide derivatives and 1D10 antibodies are administered in combination with other therapies such as chemotherapy, surgery, radiotherapy, photodynamic therapy, gene therapy, antisense therapy, enzyme prodrug therapy, immunotherapy, fusion toxin therapy, antiangiogenic therapy, or any other therapy for B-CLL or any combination of these therapies.

The duration of therapy with the pharmaceutical compositions used in the methods of the present invention will vary, depending on the unique characteristics of the pharmaceutical composition and the particular therapeutic effect to be achieved, the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. Ultimately the attending physician will decide on the appropriate duration of therapy with the pharmaceutical composition used in the method of the present invention.

Preferably, the methods of treatment described herein result in the patient being cured of the B-CLL or going into remission from the B-CLL, staying in remission and becoming a long-term survivor. However, it is not necessary that curing or remission and long-term survival be the result of the inventive treatment. Any increase in the lifespan of the patient with the inventive treatment as compared to lifespan without the inventive treatment is a desirable goal. Alternatively, effects of the inventive treatment may be measured as an improvement in the quality of life of the patient or decrease in patient suffering, absent an increase in lifespan of the patient.

EXAMPLES

The invention may be better understood by reference to the following examples which serve to illustrate but not to limit the present invention.

Example 1. Patients and Isolation of Their Peripheral Blood Mononuclear Cells

B-CLL cells were obtained from patients previously diagnosed as having B-CLL. These patients had B-CLL as defined by B.D. Cheson et al., 1996, Blood 87:4990-7. All patients with B-CLL had been without prior therapy for B-CLL for a minimum of 2 months.

Peripheral blood mononuclear cells (PBMCs) were obtained from the B-CLL patients and were also obtained from healthy volunteers as controls. Peripheral blood was collected from the patients or volunteers using standard techniques (see for example, S. Bennett and S.N. Breit, 1994, Variables in the isolation and culture of human monocytes that are of particular relevance to studies of HIV, J. Leukoc. Biol. 56:236-40). PMBCs were isolated from the blood using standard density gradient centrifugation using Ficoll-Paque Plus (Pharmacia Biotech, Picsataway NJ). After isolation, the PBMCs were washed in phosphate-buffered saline (PBS).

Such PBMC preparations comprised leukocytes, including T cells, B cells, monocytes and NK cells. PBMC preparations from B-CLL patients used in this study typically comprised 50-90% B-CLL cells compared to only a few percent of B cells in PBMC preparations from the healthy volunteers.

Example 2. LC_{50} Determination of MS-275 for B-CLL Cells

In order to accurately represent the *in vivo* situation where drug is eliminated from the bloodstream, 1 x 10^6 PBMCs from B-CLL patients or healthy volunteers, isolated as described in Example 1, were incubated in various concentrations of MS-275 in RPMI 1640 with 10% heat-inactivated fetal bovine serum for 4, 24 or 96 hours at 37°C in 96-well plates. Following incubation in MS-275, the cells were removed from media containing MS-275 and re-incubated in media without the drug. Cells that were incubated in drug for 4 hours were re-incubated in media without drug for 92 hours. Cells that were incubated in drug for 24 hours were re-incubated in media without drug for 72 hours. Cells that were incubated in drug for 96 hours were not re-incubated in media. Viability of the cells was then analyzed using the MTT assay. In this assay, cells are exposed to MTT [i.e., 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], which is taken into the cells and reduced by mitochondrial dehydrogenase to a purple formazan, a large molecule which is unable to pass through intact cell membranes, and therefore accumulates in healthy cells. The ability of cells to reduce MTT is an indication of mitochondrial integrity and activity, which is interpreted as a measure of viability. To use the MTT assay, MTT reagent was added and the cells were incubated for an additional 24 hours before washing and analysis of purple formazan formation by spectrophotometry in a

Labsystems 96-well plate reader. Data were plotted and values were calculated using GraphPad software (San Diego, CA). The data for the B-CLL cells are shown in **Figure 1**.

The results show that the LC_{50} with 4 hours of drug incubation was 1.21 μM for B-CLL cells (**Figure 1A**). In a separate experiment, the LC_{50} with 4 hours of drug incubation was at least 21.1 μM for normal cells (i.e., PBMCs isolated from individuals without B-CLL). The results in **Figure 1B** show that the LC_{50} with a continuous 96 hour drug incubation was 0.509 μM for B-CLL cells. In a separate experiment, the LC_{50} with a 96 hour continuous drug incubation was at least 4.75 μM for normal cells. These experiments were performed using cells from thirteen separate patients and four normal volunteers. These results demonstrated that MS-275 has significant and selective activity against B-CLL cells relative to normal cells.

Example 3. MS-275 Induced Histone Acetylation in CLL Cells

To determine if MS-275 caused inhibition of histone deacetylases, B-CLL cells, isolated as described in Example 1, were incubated with 0.3 or 3.0 μM of MS-275 for 24 hours and assessed for changes in histone acetylation by immunoblotting using antibodies immunospecific for acetylated H3 or H4 histones. Antibodies immunospecific for specific acetylated lysine residues at the N terminus of histone H4 (i.e., K5, K8, K12 and K16) were also used. For immunoblotting, whole cell lysates were prepared from the cells using standard procedures. Protein samples were separated along with molecular weight markers (BioRad) on 10 to 14% SDS-polyacrylamide gels. Proteins in the gels were transferred using a semi-dry apparatus (BioRad) onto nitrocellulose membranes (Schleicher and Schuell, Keene, NH). Gel loading equivalence was confirmed by Ponceau S stain (Sigma) of membranes and by probing with a monoclonal antibody for GAPDH. Blots were developed with chemiluminescent substrate (Pierce Super-Signal, Pierce Biotechnology, Rockford, IL) and autoradiography was performed with X-OMAT film (Kodak, Rochester, NY). Protein bands were digitally quantified using a ChemiDoc instrument (BioRad). The immunoblot data are shown in **Figure 2**. Quantification of these data are shown in Table 1 below.

Table 1. Fold Increase in Histone Acetylation (Relative to Untreated) after 24 Hours of MS-275 Treatment.

Patient No.	MS-275 Concentration	H3 Total	H4 Total	H4 K5	H4 K8	H4 K12	H4 K16
1	0.3 μM	1.3	2.4	2.7	1.6	4.4	3.7
	3.0 μM	2.3	3.9	8.9	1.9	15.8	9.1
2	0.3 μM	1.5	3.8	2.6	4.4	5.1	3.1
	3.0 μM	3.1	5.2	4.5	4.8	10.3	4.7

The data show that there were increases in acetylation of both histones H3 and H4 due to MS-275. Generally, the data also show that acetylation of H4 lysines K5, K8, K12 and K16 also increased due to MS-275 treatment.

Example 4. MS-275-Induced Apoptosis in B-CLL Cells

To determine whether MS-275-treated B-CLL cells were undergoing apoptosis, an annexin V/propidium iodide flow cytometric assay was used to analyze the B-CLL cells, isolated as described in Example 1, from four different patients. Staining of cells for annexin V is an early indicator of initiation of apoptosis. Staining of cells with propidium iodide is an indicator that cells are no longer viable and, therefore, that apoptosis has actually occurred (i.e., integrity of cell membrane has been breached). To perform the assay, cells were exposed to various concentrations of MS-275 for 4 hours. Then, the cells were washed, resuspended in complete medium and incubated for 24 or 48 hours in absence of MS-275. At these times, cells were washed with PBS and resuspended in binding buffer containing annexin V-FITC and propidium iodide (BD Pharmingen, San Diego, CA). After 15 minutes of incubation in the dark at room temperature, apoptosis was assessed by flow cytometry on a Coulter EPICS-XL flow cytometer. The data **(Figure 3)**, from one representative patient, show that at 3.0 μM of MS-275, apoptosis was detected within 24 hours. The studies also showed that at 0.3 μM of MS-275, apoptosis was detected after 48 hours. The results were similar in the other three patients. These data show that MS-275 induced apoptosis in B-CLL cells.

In other studies it was shown that MS-275-induced apoptosis was inhibited by expression of the anti-apoptotic protein Bcl-2, indicating that MS-275 induces apoptosis via the intrinsic (mitochondrial) pathway.

Example 5. Pathway Utilized by CLL Cells Undergoing MS-275-Induced Apoptosis

To determine the role and identity of caspases involved in MS-275-induced apoptosis, B-CLL cells, isolated as described in Example 1, were treated with MS-275 and analyzed for caspase activation by colorimetric assay. Colorimetric assay kits from R&D Systems (Minneapolis, MN) were used as suggested by the manufacturer. As shown in **Figure 4**, MS-275 induced activation of caspase 3. In other studies, it was found that caspases 8 and 9 were also activated to a lesser extent than caspase 3. Additional studies showed that the cleaved, activated forms of caspases 3, 8 and 9 were detected in B-CLL cells after treatment with MS-275. The activated caspase 3 was shown to cleave a known substrate, Poly(ADP-Ribose) Polymerase (i.e., PARP). Cleavage of the protein BID, a known substrate of caspase 8, was also shown.

Example 6. Increased Expression of 1D10 Antigen in B-CLL Cells by MS-275

Washed B-CLL cells, isolated as described in Example 1, from four different patients with B-CLL, were incubated at a concentration of 10^7 cells per ml in the presence or absence of 3.0 μM MS-275 for 24 hours, before being immunofluorescently stained with either non-specific antibody (unable to specifically bind to the cells) or antibody immunospecific for 1D10 antigen. All cells were also stained with an antibody immunospecific for CD19 (recognizing B cells). The cells were analyzed by flow cytometry. The mean fluorescence intensity due to 1D10 antigen staining of CD19-positive cells was recorded. These results are shown in Table 2 below.

Table 2. 1D10 Antigen Levels on B-CLL Cells after MS-275 Induction as Measured by Immunofluorescence Flow Cytometry

	Patient No.[1]			
Cells	1	2	3	4
Staining control[2]	1.19 (1)	1.18 (1)	1.98 (1)	1.31 (1)
No drug control[3]	69.45 (58)	145.21 (123)	2017.64 (1019)	2424.39 (2232)
MS-275[4]	104.11 (88)	463.23 (393)	6438.03 (3252)	5446.76 (4158)

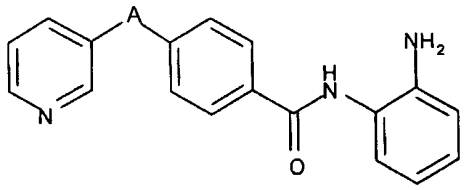
[1]Patients 1-4 represent 4 different patients with B-CLL; the numbers in the table represent values for mean 1D10 fluorescence of the cells; the numbers in parenthesis are values normalized to the staining control for each patient.
[2]B-CLL cells, no drug treatment, were stained with a non-specific antibody.
[3]B-CLL cells, no drug treatment, were stained with antibody immunospecific for 1D10 antigen.
[4]B-CLL cells, MS-275 treatment, were stained with antibody immunospecific for 1D10 antigen.

The data show that B-CLL cells expressed relatively low levels of 1D10 antigen which were induced by MS-275 treatment.

Claims

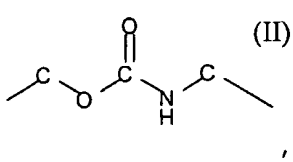
What is claimed is:

1. A method for increasing expression of 1D10 antigen in B-CLL cells in a patient with B-CLL, comprising contacting the B-CLL cells with one or more benzamide derivatives represented by the formula (I):

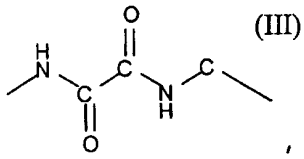


(I)

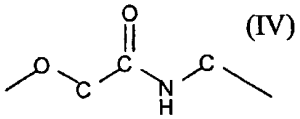
wherein A represents a structure shown by either of the formulas II, III or IV:



(II)



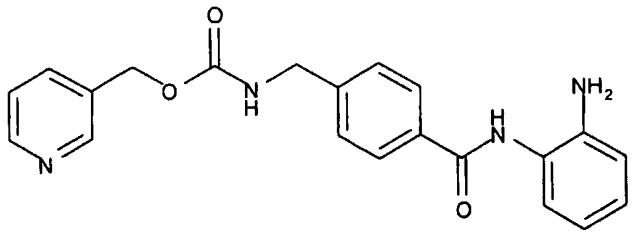
(III)



(IV)

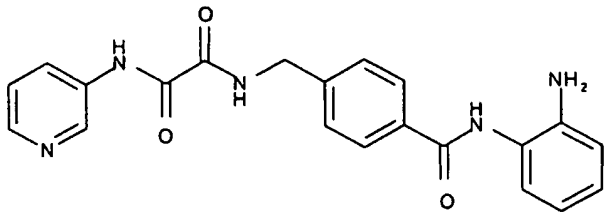
or a pharmaceutically acceptable salt thereof.

2. The method of claim 14 wherein the benzamide derivative is represented by the formula (V):



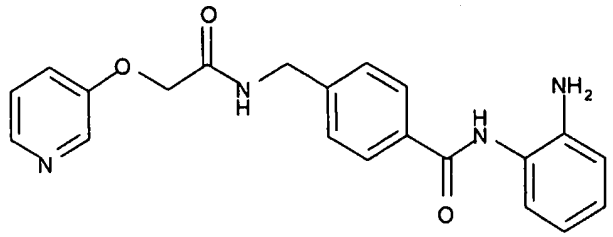
(V)

3. The method of claim 14 wherein the benzamide derivative is represented by the formula (VI):



(VI)

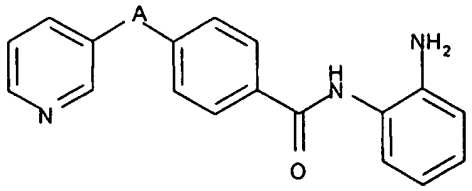
4. The method of claim 14 wherein the benzamide derivative is represented by the formula (VII):



(VII)

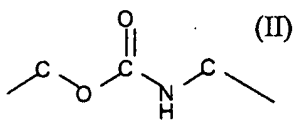
5. A method for treating a patient suspected of having or known to have chronic lymphocytic leukemia (CLL) of B cells, comprising:

administering a biologically effective amount of one or more anti-CLL agents to the patient, said agents comprising a benzamide derivative represented by the formula (I):

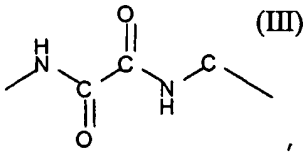


(I)

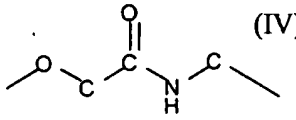
wherein A represents a structure shown by either of the formulas II, III or IV:



(II)



(III)



(IV)

or a pharmaceutically acceptable salt thereof, and;

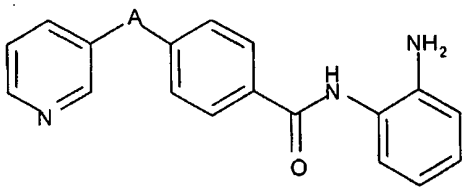
administering a biologically effective amount of one or more antibodies immunospecific for the 1D10 antigen to the patient.

6. The method of claim 7 wherein the antibodies are monoclonal antibodies.

7. The method of claim 7 wherein the antibodies are humanized monoclonal antibodies.

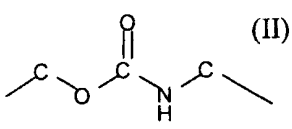
8. A method for treating a patient suspected of having or known to have chronic lymphocytic leukemia (CLL) of B cells, comprising:

administering a biologically effective amount of one or more anti-CLL agents to the patient, said agents comprising a benzamide derivative represented by the formula (I):

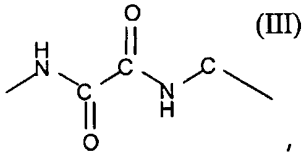


(I)

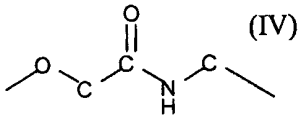
wherein A represents a structure shown by either of the formulas II, III or IV:



(II)



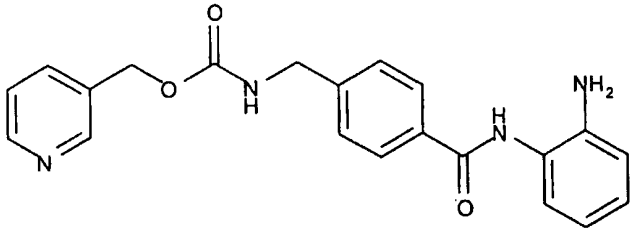
(III)



(IV)

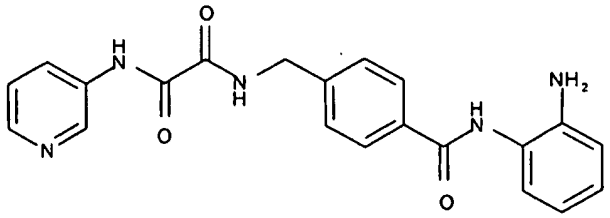
or a pharmaceutically acceptable salt thereof.

9. The method of claim 1 wherein the anti-CLL agent is represented by the formula (V):



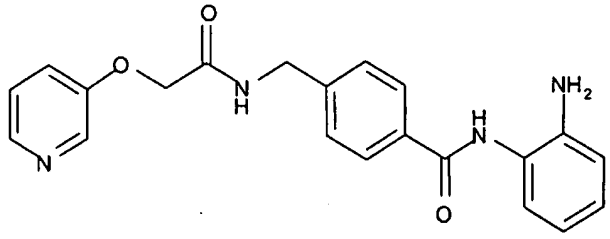
(V)

10. The method of claim 1 wherein the anti-CLL agent is represented by the formula (VI):



(VI)

11. The method of claim 1 wherein the anti-CLL agent is represented by the formula (VII):

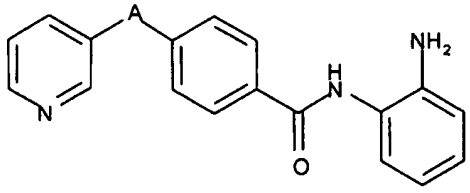


(VII)

12. The method of claim 1 wherein the anti-CLL agent is administered orally.

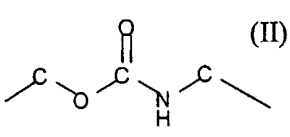
13. A pharmaceutical composition for treating a patient suspected of having or known to have chronic lymphocytic leukemia (CLL) of B cells, comprising one or more benzamide derivatives represented by formula I as in claim 1, or pharmaceutically acceptable salts thereof, and one or more components selected from the group diluents, excipients, vehicles, additives and other therapeutic agents.

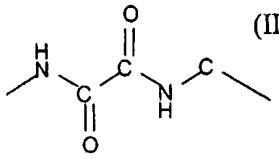
14. A method for inducing apoptosis of B-CLL cells in a patient with B-CLL, comprising contacting the B-CLL cells with one or more anti-CLL agents, said agents comprising a benzamide derivative represented by the formula (I):

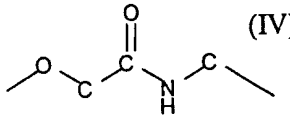


(I)

wherein A represents a structure shown by either of the formulas II, III or IV:

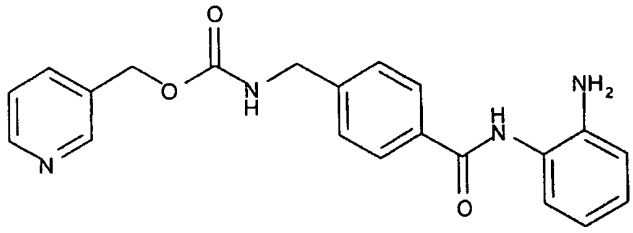

(II)


(III)

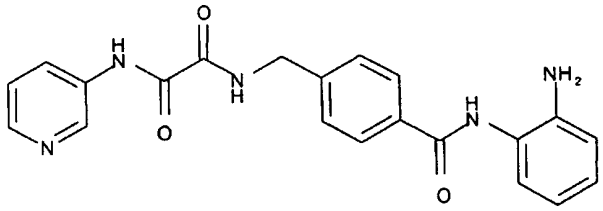

(IV)

or a pharmaceutically acceptable salt thereof.

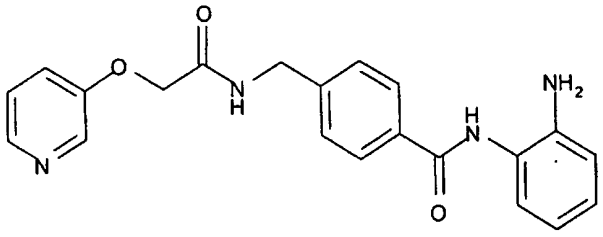
15. The method of claim 10 wherein the anti-CLL agent is represented by the formula (V):


(V)

16. The method of claim 10 wherein the anti-CLL agent is represented by the formula (VI):


(VI)

17. The method of claim 10 wherein the anti-CLL agent is represented by the formula (VII):


(VII)

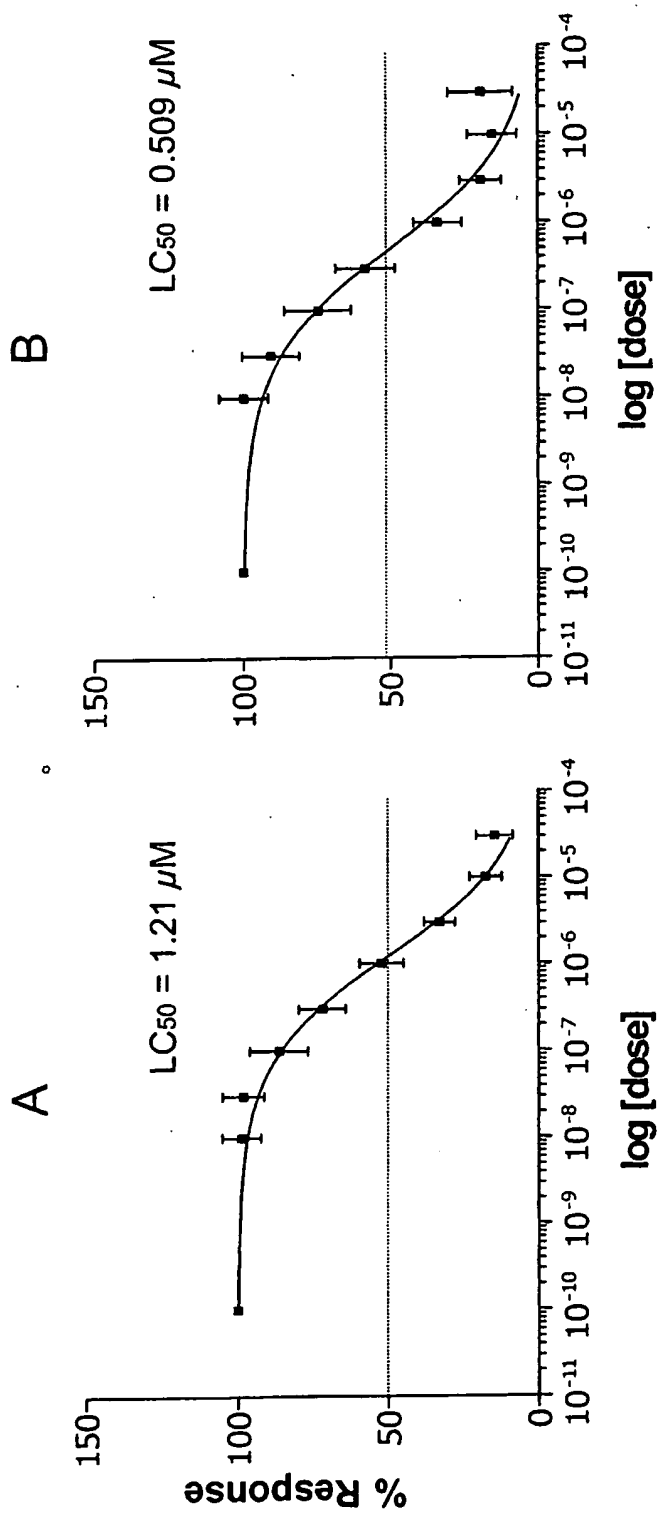

A
LC50 = 1.21 μM
B
LC50 = 0.509 μM
% Response
log [dose]
log [dose]
150
100
50
0
10-11 10-10 10-9 10-8 10-7 10-6 10-5 10-4

Figure 1

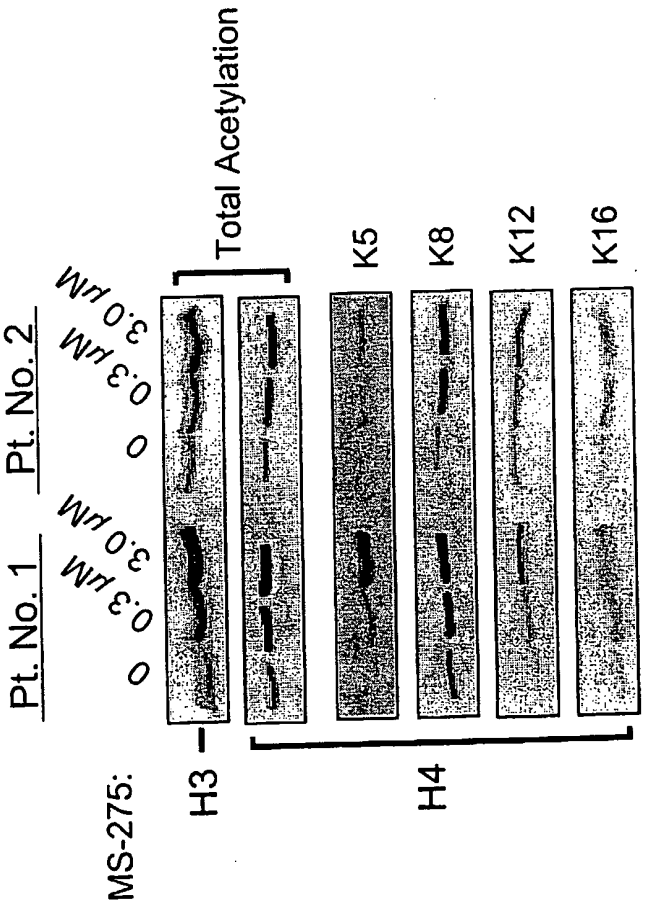

Pt. No. 1
Pt. No. 2
0
0.3 μM
3.0 μM
0
0.3 μM
3.0 μM
MS-275:
H3
H4
Total Acetylation
K5
K8
K12
K16

Figure 2

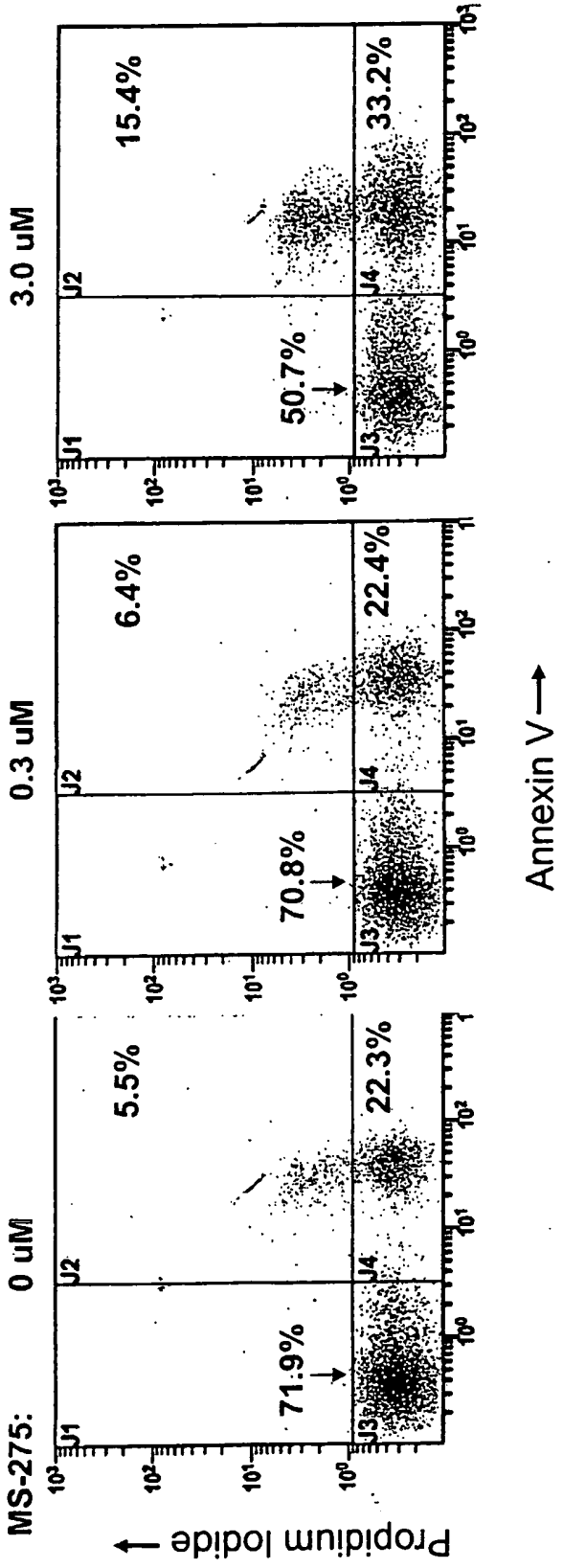
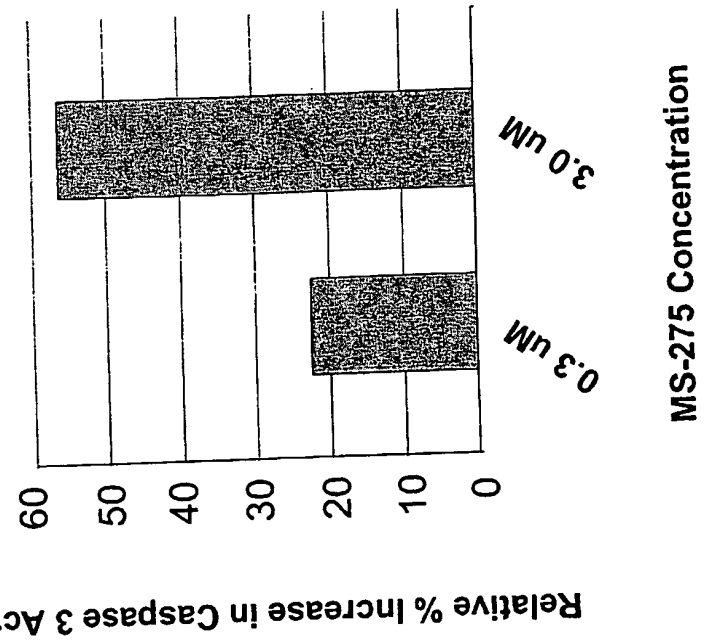

MS-275:
0 uM
0.3 uM
3.0 uM
5.5%
71.9%
22.3%
6.4%
70.8%
22.4%
15.4%
50.7%
33.2%
J1
J2
J3
J4
Propidium Iodide
Annexin V

Figure 3

Figure 4


Relative % Increase in Caspase 3 Activity
60
50
40
30
20
10
0
0.3 uM
3.0 uM
MS-275 Concentration

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/19311

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/44; A01N 25/00

US CL : 514/357,345,299,908

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/357,345,299,908

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database BIOSIS on STN, AN 2002:367448, LUCAS et al. MS-275 reduces viability and	1-2, 5-9,12-15
---	induces change inhistone acetylation in cells from patients with B-cell chronic	---
Y	lymphocytic leukemia. Proceedings of the American Association for Cancer Research Annual Meeting, March 2002, Vol. 43, pp 69, meeting abstract.	3,4,10,11,16,17
X	Database BIOSIS on STN, AN 2003:336100, LUCAS et al. MS-275 promotes apoptosis	1,2,5-9,12-15
---	in cell form patient with B-cell chronic lymphocytic leukemia (B-CLL) concurrent with	---
Y	inhibition of histone deactylase. Blood, 16 November 2002, Vol. 100, No. 11, pp. Abstract No. 1500.	3,4,10,11,16,17
Y	JP 2000256194 (SUZUKI et al.) 19 September 2000 (19.09.2000), see the Abstract.	1-17

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

19 December 2003 (19.12.2003)

Date of mailing of the international search report

10 MAY 2004

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US
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Alexandria, Virginia 22313-1450

Facsimile No. (703)305-3230

Authorized officer

Shengjun Wang

Telephone No. (703) 305-1235

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

PCT/US03/19311

Continuation of B. FIELDS SEARCHED Item 3:
CAS ONLINE, MEDLINE, BIOSIS search terms: leukemia, chronic lymphocytic leukemia, cancer, MS-275, structure search .